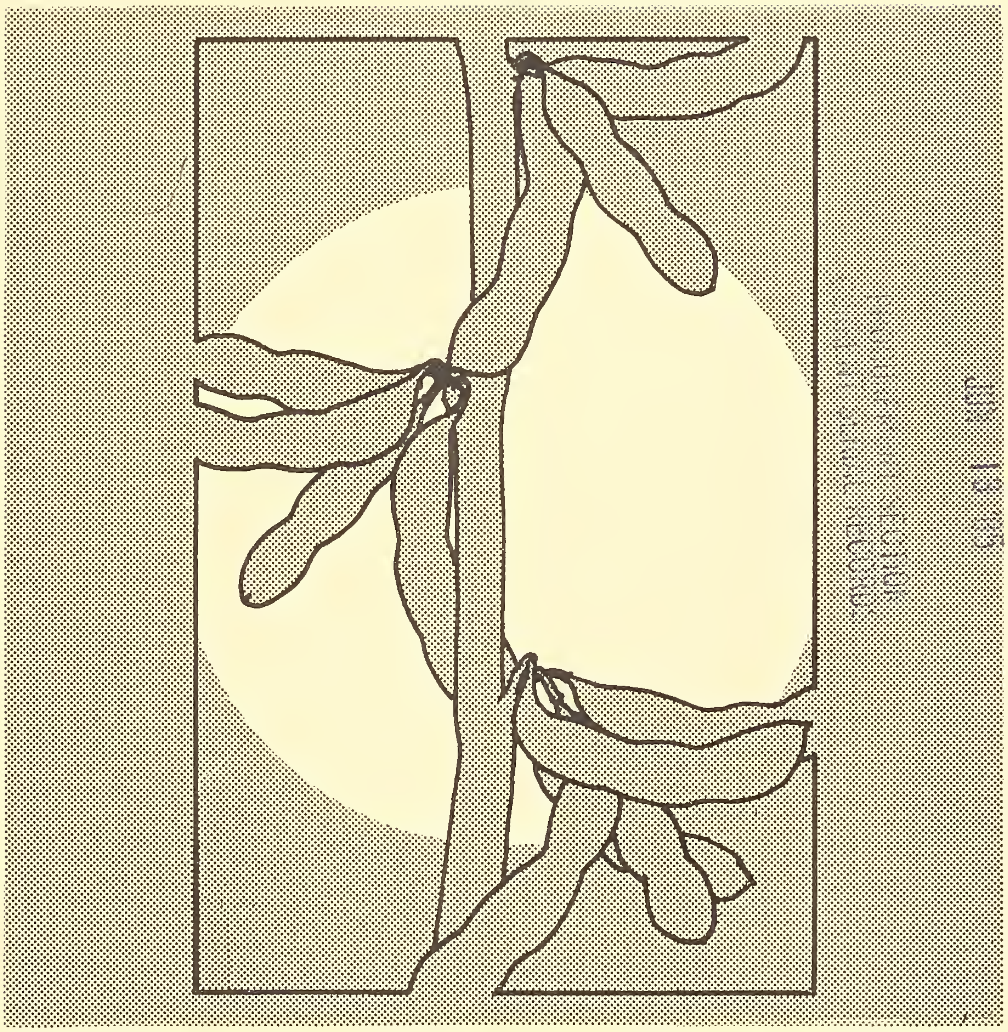
Soybean Genetics Newsletter



Volume 12 April 1985

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I. FOREWORD

The contents of this issue of the Soybean Genetics Newsletter covers a wide variety of special aspects of soybean research. Your attention is called especially to the reports of the germplasm advisory committee, and the report of the northern soybean germplasm collection. The burgeoning, in the field of soybean research, of the relatively new science of electrophoresis has necessitated a reappraisal of rules of nomenclature, and even a renaming of some of the genes whose existence has been verified by electrophoresis. This year, the soybean genetics committee discussed and decided on changes in nomenclature. Their report is to be found in this issue, with the rule changes underlined.

Graduate students and technicians who volunteered (or were drafted) to help in the assembly and publication of Volume 12 of the Soybean Genetics Newsletter are: Lon-Fang O. Chen, Jeff Griffin, Peggy Hatfield, Brad Hedges, Huang Jintai, Phyllis Tyrrell, Diane Stevermer, Cyndi Lee, Duane Garien, Peggy Thorson, Mary McFerson, Holly Heer and Susan Yost. It would be a mighty thin volume without their help.

Reid G. Palmer, editor

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II. ANNOUNCEMENTS

"An ultrastructural study of soybean seed development," a 36-page pamphlet by Shong Wan Norby, Clifford A. Adams and Robert W. Rinne, is available upon request from the authors. Twenty pages are devoted to electron micrographs of soybean cotyledon cells. Data on protein, oil, and starch development, on seed moisture and seed dry weight during seed development are presented in graph form. The research was supported in part by the American Soybean Association. R. E. Rinne is plant physiologist with the Agricultural Research Service, United States Department of Agriculture, Department of Agronomy, 1102 S. Goodwin Avenue, University of Illinois, Urbana, IL 61801.

A NOTE SOLICITING FOR SUBSCRIPTIONS TO JOURNAL "SOYBEAN SCIENCE"

The magazine "Soybean Science" sponsored by the Soybean Institute, Heilongjiang Academy of Agricultural Sciences, is openly published as one of the national scientific periodicals at home and abroad. It is a quarterly with about 80 pages and 16 mo in size and cost $60 a year (including postage).

The main content in this magazine is the papers, notes, reviews and news in brief about genetics, plant breeding, plant physiology, ecology, germplasm resources, the control of diseases, insects and weeds, nutrition, application of fertilizers and biology of soybean.

The "Soybean Science" is available to all scientific researchers at home or abroad, teachers and students in agriculture colleges and scientific workers in farms, ranches and agricultural extension services.

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O R D E R

Name of Magazine	Cost (per year)	Quantity	Sum
Soybean Science	$60		

Unit (or person)____

Person Handling a Transaction________________________________

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NOTES:

1. Please mail directly the Check or money order to Editorial Department of Soybean Science Magazine, Heilongjiang Academy of Agricultural Sciences, Harbin, People's Republic of China (postage paid by subscriber).
2. Please mail the Check or money order to Editorial Department before end month year, so that we will arrange printing.

III. SOYBEAN GENETICS COMMITTEE REPORT

Minutes of the Meeting

The Soybean Genetics Committee met Feb. 25, 1985, at the Ramada Inn - Executive Plaza, Memphis, TN. This meeting was held in conjunction with the Annual Soybean Breeders Workshop.

Committee members in attendance were W. D. Beversdorf, R. L. Bernard, H. R. Boerma, X. Delannay, T. C. Kilen, J. H. Orf, R. G. Palmer, and J. R. Wilcox. Also present were J. Griffin, B. Hedges, T. Hymowitz and Y. T. Kiang. R. I. Buzzell and X. Delannay have been elected to new three-year terms on the committee replacing Kilen and Orf, whose terms expired at the close of the meeting. Present committee members and the expiration of their terms are as follows:

R. L. Bernard, Ex officio
(Curator of soybean genetics collection)
Department of Agronomy
University of Illinois
1102 S. Goodwin St.
Urbana, IL 61801

W. D. Beversdorf, Chairman (1987)
Crop Science Department
University of Guelph
Guelph, Ontario
Canada N1G 2W1

H. R. Boerma (1986)
Department of Agronomy
University of Georgia
Athens, GA 31794

R. I. Buzzell (1988)
Agriculture Canada, Research Station
Harrow, Ontario
Canada NOR 1GO

T. E. Devine (1986)
Rm. 218, Bldg. 001
BARC-West
Beltsville, MD 20705

R. G. Palmer, Ex officio
(Editor of Soybean Genetics Newsletter)
Department of Agronomy
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J. R. Wilcox (1987)
Department of Agronomy
Purdue University
West Lafayette, IN 47907

X. Delannay (1988)
Monsanto Agricultural Products Company
700 Chesterfield Village Parkway
Mail Zone GG4A, St. Louis, MO 63198

W. D. Beversdorf was elected chairman of the committee for 1985, so manuscripts concerning qualitative genetic interpretation and gene symbols should be sent to him for review.

The number of manuscripts received for review by the committee increased from 18 to 20. Persons who are not members of the committee may be asked to review manuscripts when their area of expertise is needed and/or to spread the workload.

A number of changes in the rules for genetic symbols were approved by the Committee. The major changes were revisions to accommodate the optional use of gene symbols on one line (the use of subscripts and superscripts is still permitted) and amendments dealing with isoenzyme and protein gene symbols. These amendments were made since several recent articles, both in the Soybean Genetics Newsletter and elsewhere, have reported on the inheritance of isoenzyme variants in soybeans. Some of these publications have assigned gene symbols to the loci responsible for these variants. There is a lack of consistency in the nomenclature used by the various research groups responsible for this work. In order to avoid confusion in the future, the committee decided it was necessary to agree on a common system of gene symbol nomenclature.

The changes in the gene symbol rules approved by the committee are underlined in the rules published this year.

The committee discussed the type and amount of data needed for the assignment of gene symbols. The requirements will be published in the Soybean Genetics Newsletter so that those researchers submitting articles with the assignment of new gene symbols will know what data are expected before the committee will consider assigning a gene symbol.

A committee consisting of W. Beversdorf, X. Delannay, Y. T. Kiang and T. Hymowitz was appointed to consider rules for gene symbols introduced into soybeans from other organisms via such methods as gene transfer, transformation, and other genetic engineering techniques and also to consider rules for assigning gene symbols to genes in the perennial species of the subgenus *Glycine*. A report and/or proposal will be made at the 1986 committee meeting.

The committee also discussed the use of provisional gene symbols and the reassignment of gene symbols. After some discussion, it was agreed that T-numbers instead of provisional symbols would be appropriate until the genetics of a new genotype were completed. The committee also agreed not to change the $\underline{Sp_1}$ gene symbol (the symbol for beta-amylase).

The committee urges researchers who report lines carrying new genes to submit a seed sample to R. L. Bernard so a genetic type collection designation (T-number) can be assigned. Dr. Bernard will maintain the seed and have it available on request.

Rules for Genetic Symbols

I) Gene Symbols

a) Gene symbols should not be assigned to traits for which no inheritance data are presented.

b) A gene symbol shall consist of a base of one to three letters, to which may be appended subscripts and/or superscripts as described below. Gene symbols may, however, be written on one line.

c) Genes that are allelic shall be symbolized with the same base letter(s) so that each gene locus will be designated by a characteristic symbol base.

d) Gene pairs with the same or similar effects (including duplicate, complementary, or polymeric genes) should be designated with the same letter base differentiated by numerical subscripts, assigning 1, 2, 3, 4, etc., consecutively in the order of publication. (Example: Y_1, Y_2, etc.) The numerals may be written on the same line as the base. (Example: Y1, Y2, etc.) This shall be the only use of numerals. Letter designations should not be used. The numeral 1 is automatically a part of the first reported gene symbol for each base but may be omitted only until the second symbol is assigned.

3) The first pair of alleles reported for a gene locus shall be differentiated by capitalizing the first letter of the symbol for the dominant or partially dominant allele. (Example: Ab, ab. Ab is allelic and dominant to ab.)

f) If two alleles are equivalent, codominant, or if dominance is not consistent, the capitalized symbol may be assigned at the author's discretion and the alleles may be differentiated by adding one or two uncapitalized letters as superscripts to the base. When more than two alleles exist for a locus, the additional alleles or those symbolized subsequently to the pair first published shall be differentiated by adding one or two uncapitalized letters as a superscript to the base. (Example: R, r^m, r.) This shall be the only use of superscripts. The letters may be written on the same line as the base if preceded by a hyphen. (For example Rpsl-b, Rpsl-k, and Ap-a, Ap-b, Ap-c). The base for the additional alleles is capitalized only when the gene is dominant or equivalent to the allele originally designated with a capitalized symbol. The letters may be an abbreviation of a descriptive term.

g) Base letters may be chosen so as to indicate apparent relationships among traits by using common initial letters for all loci in a related group of traits. Examples are P for pubescence type, R for disease reaction (plus two initials of the pathogen to complete the base), and L for leaf shape.

h) The distinction between traits that are to be symbolized with identical, similar, or with unrelated base letters is necessarily not clear cut. The decision for intermediate cases is at the discretion of the author but should be in accordance with previous practices for the particular type of trait.

The following sections concern supplementary symbols that may be used whenever desired as aids to presentation of genetic formulas.

i) A dash may be used in place of a gene symbol to represent any allele at the indicated locus. The locus represented should be apparent from its position in the formula. (Example: A_ represents both AA and Aa.)

j) A question mark may be used in place of a symbol when the gene is unknown or doubtful, or it may be used as a superscript or on the base line if preceded by a hyphen. (Example: $a^?$ or a-? indicates that the latter is an unknown allele at the A locus.)

k) Plus symbols may be used in place of the assigned gene symbols of a designated standard homozygous strain when this will facilitate presenting genetic formulas. The standard strain may be any strain selected by the worker, as long as the strain being used and its genetic formula are made explicit.

II) Isoenzyme Symbols and Protein Gene Symbols

The following set of guidelines is to be used when assigning gene symbols to isoenzyme variants. As far as possible, these recommendations are consistent with the existing guidelines for assigning gene symbols in soybeans.

a) A gene symbol (generally three letters) that indicates, as clearly as possible, the name of the enzyme should be used. For example, Adh (alcohol dehydrogenase); Idh (isocitrate dehydrogenase). The appropriate Enzyme Commission name and number should be used in the original article, when appropriate, to designate the specific enzyme activity being investigated.

b) <u>The electrophoretic conditions used to characterize a locus or allele should be specified clearly and in sufficient detail to be repeated by others interested in using the locus in genetic studies. The electrophoretic mobility, or other properties of an allele, should be clearly described by the authors</u>.

c) <u>Publications should include a photograph and/or an interpretive zymogram that allows readers to visualize the variability described by the authors, as well as to confirm that subsequent work corresponds to the original study</u>.

III) Linkage and Chromosome Symbols

a) Linkage groups and the corresponding chromosomes shall be designated with Arabic numerals. Linkage shall be indicated in a genetic formula by preceding the linked genes with the linkage group number and listing the gene symbols in the order that they occur on the chromosome.

b) <u>Permanent symbols</u> for chromosomal aberrations shall include a symbol denoting the type of aberration plus the chromosome number(s) involved. Specific aberrations involving the same chromosome(s) shall be differentiated by a letter as follows: The symbol Tran shall denote translocations. Tran 1-2a would represent the first case of reciprocal translocations between chromosomes 1 and 2, Tran 1-2b the second, etc. The symbol Def shall denote deficiencies, Inv inversions, and Tri primary trisomics. The first published deficiency in chromosome 1 shall be symbolized as Def 1a, the second as Def 1b, etc. The first published inversion in chromosome 1 shall be denoted as Inv 1a, etc. The first published primary trisomic shall be designated with the Arabic numeral that corresponds to its respective linkage group number.

c) <u>Temporary symbols</u> for chromosomal aberrations are necessary, as it may be many years before they are located on their respective chromosomes. Tran 1 would represent the first case of a published reciprocal translocation; Tran 2, the second case, etc. The first published deficiency shall be symbolized as Def A, the second as Def B, etc. The first published inversion shall be symbolized as Inv A, and second as Inv B. The first published trisomic shall be designated as

Tri A, the second as Tri B, etc. When appropriate genetic and/or cytological evidence is available, the temporary symbols should be replaced with permanent symbols, with the approval of the Soybean Genetics Committee

IV) Cytoplasmic Factor Symbols

a) Cytoplasmic factors shall be designated with one or more letters prefixed by cyt-. (Example: cyt-G indicates the cytoplasmic factor for maternal green cotyledons, cyt-Y indicates that for maternal yellow cotyledons).

V) Priority and Validity of Symbols

a) A symbol shall be considered valid only when published in a recognized scientific journal, or when reported in the Soybean Genetics Newsletter, with conclusions adequately supported by data which establish the existence of the entity being symbolized. Publication should include an adequate description of the phenotype in biological terminology, including quantitative measurements wherever pertinent.

b) In cases where different symbols have been assigned to the same factor, the symbol first published should be the accepted symbol, unless the original interpretation is shown to be incorrect, the symbol is not in accordance with these rules, or additional evidence shows that a change is necessary.

VI) Rule Changes

a) These rules may be revised or amended by a majority vote of the Soybean Genetics Committee.

It is recommended that all gene symbols and genetic interpretation be reviewed by the Soybean Genetics Committee prior to publication to avoid duplication and/or confusion.

IV. SOYBEAN GERMPLASM ADVISORY COMMITTEE REPORT

The Soybean Germplasm Advisory Committee held its annual meeting Feb. 26, 1985, at the Soybean Breeders' Workshop in Memphis, Tennessee. Twelve of the 14 committee members were in attendance. The committee reviewed developments regarding soybean germplasm exchange with the Peoples' Republic of China. Very little progress had been made during the year, and it will continue to be an issue of concern for the committee. Election procedures, used for the first time this year, were reviewed. No major changes were suggested, but efforts to involve more of the soybean germplasm user community are needed. Those elected to three-year terms were: James Orf, University of Minnesota, Reid Palmer, USDA, Iowa State University, and Michael Sullivan, Clemson University.

Updates on both the northern and southern portions of the USDA Soybean Germplasm Collection were given by Richard Bernard and Calton Edwards, respectively. Those reports are presented elsewhere in this volume, so no details will be given here. The committee was briefed on the possible changes in assignment for personnel associated with the northern germplasm collection. The committee expressed concern about the effect that those changes would have on current efforts to expand the collection. The committee unanimously passed a motion urging the USDA to move forward as rapidly as possible to arrange for germplasm exchanges with collections around the world recently identified in a joint International Board of Plant Genetic Resources, International Soybean Project of the University of Illinois, and USDA project, and to provide adequate funding for the collection, maintenance, and evaluation of that material. Data from approximately 2900 and 2200 accessions from the southern and northern collections, respectively, have now been entered into the Germplasm Resources Information Network (GRIN). Corrections are now being made and it is anticipated that public access to that data through GRIN will be available in the near future.

The status and future of the perennial *Glycine* collection was discussed. The current USDA collection of less than 100 accessions is not large enough to meet the increasing demand fueled by a growing interest in these species; however, current facilities are not adequate to maintain the large number of accessions that could be obtained from Australia and the University of Illinois. A sub-committee of Richard Bernard, Reid Palmer, and Curtis Williams was

appointed to study the staffing and facilities alternatives discussed and to make a recommendation to the committee.

Randall Nelson reported that germplasm evaluation data he has collected and compiled with the help of Jean Lambert and James Orf, University of Minnesota, for over 2000 soybean accessions were distributed at the Soybean Breeders' Workshop. This information is currently available in three reports: maturity groups 0 and earlier, maturity groups I, II and III, and early maturing accessions in maturity group IV. For all groups, the data include all accessions between PI 273483 and PI 427107. These data will also be entered into GRIN this spring. Data on the remainder of the maturity group IV accessions with PI numbers less than 427107 will be available early this summer and data on approximately 2000 accessions in maturity groups 000 through IV between PI 427107 and PI 445845 will be available by September.

The future direction of the committee was discussed. It was decided that no major changes were needed, but that a yearly updated list of germplasm needs and research priorities would be sent to the appropriate USDA administrators each fall. Input to the committee from the germplasm user community is always welcome. Following are the current committee members, addresses, area of representation, and date of expiration of current term:

Name	Address	Area of Representation	Expiration of Term
R. L. Bernard	USDA ARS and Agronomy University of Illinois 1102 South Goodwin Urbana, IL 61801	USDA Germplasm Collection	ex officio
Edgar E. Hartwig	Delta Branch Exp. Stn. Box 196 Stoneville, MS 38776	USDA Germplasm Collection	ex officio
Thomas C. Kilen	USDA ARS SR P.O. Box 196 Stoneville, MS 38776	USDA Germplasm Collection	ex officio
Randall Nelson	USDA ARS and Agronomy University of Illinois 1102 South Goodwin Urbana, IL 61801	USDA Germplasm Collection	ex officio
Phillip Miller	USDA Beltsville Agric. Res. Center Building 005, BARC-West Beltsville, MD 20705	USDA National Program Staff	ex officio

Name	Address	Area of Representation	Expiration of Term
Kuell Hinson	USDA ARS and Agronomy University of Florida 304 Newell Hall Gainesville, FL 32611	Public Breeding, South	1987
Clark Jennings	Pioneer Hi-Bred Int'l P.O. Box 854 Cedar Falls, IA 50613	Private Breeding, North	1987
Curtis Williams	Jacob Hartz Seed Co. Box 946 Stuttgart, AR 72160	Private Breeding, South	1986
S. M. Lim	USDA ARS and Plant Path. University of Illinois 1102 South Goodwin Urbana, IL 61801	Pathology	1986
R. A. Kinlock	Agricul. Research Ctr. Route 3, Box 575 Jay, FL 32565	Nematology	1987
M. J. Sullivan	Edisto Experiment Stn. P.O. Box 247 Blackville, SC 29817	Entomology	1988
Richard Wilson	4124 Williams Hall N. Carolina State Univ. Raleigh, NC 27650	Physiology	1986
Reid G. Palmer	USDA ARS 4 Curtiss Hall Iowa State University Ames, IA 50011	Cytogenetics and Molecular Genetics	1988
J. H. Orf	Dept. of Agronomy and Plant Genetics University of Minnesota St. Paul, MN 55101	Plant Breeding, North	1988

Thomas Kilen was elected as chairperson of the committee and Clark Jennings was elected vice-chair. Both will serve one-year terms.

Randall Nelson, Chairperson
Soybean Germplasm Advisory Committee

V. U.S. NORTHERN SOYBEAN GERMPLASM COLLECTION REPORT

The 1984 summary of the USDA Soybean Germplasm Collection at Urbana, Illinois, is as follows:

Total number of soybean accessions by maturity group:

Maturity group	Old varieties	FC strains	PI strains 1984 additions	PI strains Total	Total	Percentage
000	3	1	0	89	93	1.3
00	5	4	3	330	339	4.8
0	7	6	4	823	836	11.7
I	23	3	3	1111	1137	16.0
II	26	6	5	1182	1214	17.0
III	38	13	0	1074	1125	15.8
IV	38	18	20	2322	2378	33.4
Total	140	51	35	6931	7122	100.0

Total number of soybean accessions by country of origin:

Country of origin	Old varieties	FC strains	PI strains 1984 additions	PI strains Total	Total	Percentage
China	64	4	2	1210	1278	17.9
Japan	34	10	0	1034	1078	15.1
Korea	12	0	4	2039	2051	28.8
USSR	6	0	12	1810	1816	25.5
Other Asian	0	0	17	34	34	.5
Europe	3	0	0	758	761	10.7
US/Canada	20	36	0	0	56	.8
Other*	1	1	0	15	17	.3
Unknown	0	0	0	31	31	.4
Total	140	51	35	6931	7122	100.0

*Africa, Australia, and Latin America.

There were 40 additions to the Wild Soybean Germplasm Collection, all from China. There are now 638 accessions of wild soybean, ranging from Maturity Group 000 to X, 107 from China (17%), 183 from Japan (29%), 314 from South Korea (49%), and 34 from the Soviet Union (5%).

Checklists of U.S. and Canadian named varieties including maturity group and descriptive codes are available from the curator (Germplasm Variety Checklist, 140 strains, January 1982 and Public Variety Checklist, 149 strains, August 1984).

A new FC and PI strain checklist (6932 strains, February 1985) including maturity group is available.

A new wild soybean list (638 strains, January 1985) including maturity group and collection site information is available.

An Inventory of the USDA Soybean Germplasm Collection will be published in 1985. This Inventory includes all strains up to PI 476000, Maturity Groups 000 to X, and information on the country of origin and variety name. Copies of this publication will be available from the curator.

An International Directory of Soybean Germplasm Collections will be published in early 1985 in conjunction with the International Board for Plant Genetic Resources (IBPGR) and the International Soybean Program (INTSOY). This Directory includes information on 79 soybean germplasm collections (cultivated, wild, and perennial species) in 43 countries. Copies of this publication will be available from INTSOY, Urbana, Illinois.

R. L. Bernard, Curator
G. A. Juvik, Assistant Curator

COMMERCIAL SOYBEAN BREEDERS BOARD -- 1985

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VI. CURRENT AND OBSOLETE GENE SYMBOLS FOR ISOZYMES AND PROTEIN VARIANTS IN SOYBEAN

There has been a proliferation of reports describing isoenzymes and proteins in the genus Glycine. Guidelines for gene symbols have been revised recently by the Soybean Genetics Committee (see pages 4-9 of this volume). Table 1 lists the gene designation, phenotype, strain, and reference for currently used gene symbols. Table 2 lists the gene designation, reference, and synonymy for obsolete gene symbols.

Reid G. Palmer, USDA
Jeffrey D. Griffin
Bradley R. Hedges
Iowa State University

Table 1-Genes controlling inheritance of isoenzyme and protein variants in soybean

Gene	Phenotype	Strain*	References
Ap-a	Acid phosphatase mobility variant	Ebony	Gorman & Kiang, 1977; Hildebrand et al., 1980
Ap-b	Acid phosphatase mobility variant	Amsoy 71, c	
Ap-c	Acid phosphatase mobility variant	Earlyana, Manchu	
Adh1	Alcohol dehydrogenase present	Altona, Wilson	Gorman & Kiang, 1978; Kiang & Gorman, 1983
adh1	Alcohol dehydrogenase absent	A-100, Lindarin	
Adh2	Alcohol dehydrogenase present	Amsoy, Beeson	
adh2	Alcohol dehydrogenase absent	Cayuga, Grant	
Amy1	α-amylase band 1 present	Harosoy, Clark	Gorman & Kiang, 1977, 1978; Kiang, 1981
amy1	α-amylase band 1 absent	Altona***, PI 132201	
Amy2	α-amylase band 2 present	Harosoy, Clark	
amy2	α-amylase band 2 absent	Altona***, PI 132201	

Sp1-a	β-amylase mobility variant	Amsoy, Evans	Larsen, 1967; Larsen & Caldwell, 1968; Orf & Hymowitz, 1976; Gorman & Kiang, 1977, 1978; Hymowitz et al., 1979; Hildebrand & Hymowitz, 1980a, b; Kiang, 1981
Sp1-b	β-amylase mobility variant	Williams, Century, c	
Sp1-an	Seed protein band present, β-amylase activity weak or absent	Chestnut	
sp-1	Seed protein band absent, β-amylase activity absent	Altona***, PI 132201	
Cgy	β-conglycinin subunit α' present	c	Kitamura et al., 1984
cgy1	β-conglycinin subunit α' absent	Keburi	
Dia1	Diaphorase present	Evans, Elton	Gorman et al., 1983; Kiang & Gorman, 1983
dia1	Diaphorase (some bands absent or weak)	Cayuga, Kingston	
Dia2-a	Diaphorase mobility variant	Amsoy, Elton	
Dia2-b	Diaphorase mobility variant	Wilson, Kingston	
Dia3	Diaphorase present	Kingston	
dia3	Diaphorase absent	Elton	
Ep	High peroxidase activity	Harosoy 63	Buzzell & Buttery, 1969
ep	Low peroxidase activity	Blackhawk	
Eu	Urease fast band	Blackhawk, Chippewa 64	Buttery & Buzzell, 1971
eu	Urease slow band	Corsoy, Midwest	

Gpd	Glucose-6-phosphate dehydrogenase present	Amsoy, Evans	Gorman et al., 1983; Kiang & Gorman, 1983
gpd	Glucose-6-phosphate dehydrogenase (weak)	Chestnut, Cayuga	
Gy4	Glycinin subunit $A_5A_4B_3$ present	c	Kitamura et al., 1984
gy4	Glycinin subunit $A_5A_4B_3$ absent	Raiden	
Idh1-a	Isocitrate dehydrogenase mobility variant	Amsoy, Cayuga	Yong et al., 1981, 1982; Gorman et al., 1983; Kiang & Gorman, 1983
Idh1-b	Isocitrate dehydrogenase mobility variant	Wilson, Evans	
Idh2-a	Isocitrate dehydrogenase mobility variant	Amsoy, Cayuga	
Idh2-b	Isocitrate dehydrogenase mobility variant	Wilson, Evans	
Idh3-a	Isocitrate dehydrogenase mobility variant	Elton, Amsoy	
Idh3-b	Isocitrate dehydrogenase mobility variant	Agate, Wilson	
Lap1-a	Leucine aminopeptidase mobility variant	Norredo, Wilson	Gorman et al., 1982a, b; 1983
Lap1-b	Leucine aminopeptidase mobility variant	Lindarin,	
Lap2	Leucine amino-peptidase present	Amsoy	Kiang et al., 1984
lap2	Leucine amino-peptidase absent	Jefferson	
Le	Seed lectin present	Harosoy	Pull et al., 1978; Orf et al., 1978; Stahlhut & Hymowitz, 1980
le	Seed lectin absent	T102	

Lx1	Lipoxygenase-1 present	Harosoy, Clark	Hildebrand & Hymowitz, 1981, 1982
lx1	Lipoxygenase-1 absent	Kedelee No. 367 (PI 133226), PI 408251	
Lx3	Lipoxygenase-3 present	Raiden, Century	Kitamura et al., 1983
lx3	Lipoxygenase-3 absent	Wase Natsu (PI 417458), I-Higo-Wase, (PI 205085)	
Mpi-a	Mannose-6-phosphate isomerase mobility variant	Wilson, PI 65549 (wild soybean)	Gorman et al., 1983; Kiang & Gorman, 1983
Mpi-b	Mannose-6-phosphate isomerase mobility variant	Amsoy, Kingston	
Mpi-c	Mannose-6-phosphate isomerase mobility variant	Elton, Hark	
Pgd-a	Phosphogluconate dehydrogenase mobility variant	Agate, Kingston	Gorman et al., 1983; Kiang & Gorman, 1983
Pgd-b	Phosphogluconate dehydrogenase mobility variant	Elton, Hill	
pgd	Phosphogluconate dehydrogenase absent	Hidaka-1 (PI 406684), PI 65549 (wild soybean)	
Pgi-a	Phosphoglucose isomerase mobility variant	PI 135624 (wild soybean), PI 65549 (wild soybean)	Gorman et al., 1983; Kiang & Gorman, 1983
Pgi-b	Phosphoglucose isomerase mobility variant	Beeson, Hark	

Pgm1-a	Phosphoglucomutase mobility variant	Chestnut, Wells	Gorman et al., 1983; Kiang & Gorman, 1983
Pgm1-b	Phosphoglucomutase mobility variant	Amsoy, Hark	
Pgm2-a	Phosphoglucomutase mobility variant	PI 423990 (wild soybean), Shirosaya 1, (PI 423955)	
Pgm2-b	Phosphoglucomutase mobility variant	Amsoy, Wells	
Sod	Superoxide dismutase bands 4 and 5 present	c	Gorman & Kiang, 1978; Gorman et al., 1982b; Gorman et al., 1984; Griffin and Palmer, 1984
sod	Superoxide dismutase bands 4 and 5 absent	Evans	
Sp1**			
Ti-a	Kunitz trypsin inhibitor mobility variant	Harosoy, Clark	Singh et al., 1969; Hymowitz & Hadley, 1972; Orf & Hymowitz, 1977, 1979
Ti-b	Kunitz trypsin inhibitor mobility variant	Aoda	
Ti-c	Kunitz trypsin inhibitor mobility variant	PI 86084	
ti	Kunitz trypsin inhibitor absent	Kin-du (PI 157440), Baik Tae (PI 196168)	

*c Indicates that the gene commonly occurs in many cultivars.

**See β-amylase which follows amy2.

***Altona is a mixture of several genotypes.

Table 2 - Gene symbols that have been used and published for inheritance of isoenzyme and protein variants in soybean

Gene symbol	Reference and synonymy
Adh1,adh1	Table 1.
Adh2,adh2	Table 1.
Adh1-+,adh1-n	Kiang & Gorman, 1983. = Adh1,adh1 (Table 1).
Adh4-+,adh4-n	Kiang & Gorman, 1983. = Adh2,adh2 (Table 1).
Am1-+,am1-n	Kiang, 1981; Kiang & Gorman, 1983. = Amy1,amy1 (Table 1).
Am2-+,am2-n	Kiang, 1981; Kiang & Gorman, 1983. = Amy2,amy2 (Table 1).
Am3-s,Am3-f,Am3-sw, am3-n1	Kiang, 1981; Kiang & Gorman, 1983. = Sp1-a,Sp1-b, Sp1-an,sp1 (Table 1).
Amy1,amy1	Table 1.
Amy2,amy2	Table 1.
Ap-a,Ap-b,Ap-c	Table 1.
Cgy1,cgy1	Table 1.
Di1,di1	Gorman et al., 1983. = Dia1,dia1 (Table 1).
Di2-s,Di2-f	Gorman et al., 1983. = Dia2-a,Dia2-b (Table 1).
Di3,di3	Gorman et al., 1983. = Dia3,dia3 (Table 1).
Dia1-+,dia1-n	Kiang & Gorman, 1983. = Dia1,dia1 (Table 1).
Dia2-s,Dia2-f	Kiang & Gorman, 1983. = Dia2-a,Dia2-b (Table 1).
Dia3-+,dia3-n	Kiang & Gorman, 1983. = Dia3,dia3 (Table 1).
Ep,ep	Table 1.

Eu,eu	Table 1.
Eu1-a,Eu1-b	Kloth & Hymowitz, 1985. = Eu,eu (Table 1).
Gpd,gpd	Table 1.
Gy4,gy4	Table 1.
Idh1-a,Idh1-b	Table 1.
Idh2-a,Idh2-b	Table 1.
Idh3-a,Idh3-b	Table 1.
Idh1-s,Idh1-f	Kiang & Gorman, 1983. = Idh1-a,Idh1-b (Table 1).
Idh2-s,Idh2-f	Kiang & Gorman, 1983. = Idh2-a,Idh2-b (Table 1).
Idh3-s,Idh3-m	Kiang & Gorman, 1983. = Idh3-a,Idh3-b (Table 1).
Lap1-a,Lap1-b	Table 1.
Lap2,lap2	Table 1.
Lap1-s,Lap1-f	Gorman et al., 1982a; Gorman et al., 1983; Kiang & Gorman, 1983. = Lap1-a,Lap1-b, (Table 1).
Le,le	Table 1.
Lx1,Lx3,lx1,lx3	Table 1.
Mpi-a,Mpi-b,Mpi-c	Table 1.
Mpi-s,Mpi-m,Mpi-f	Gorman et al., 1983; Kiang & Gorman, 1983. = Mpi-a,Mpi-b,Mpi-c (Table 1).
Pgd-a,Pgd-b,pgd	Table 1.
Pgd-s,Pgd-f,pgd-n	Kiang & Gorman, 1983.= Pgd-a,Pgd-b,pgd (Table 1).
Pgi-a,Pgi-b	Table 1.
Pgi-s,Pgi-f	Kiang & Gorman, 1983. = Pgi-a,Pgi-b (Table 1).
Pgm1-a,Pgm1-b	Table 1.
Pgm1-s,Pgm1-f	Kiang & Gorman, 1983. = Pgm1-a,Pgm1-b (Table 1).
Pgm2-p,Pgm2-n	Kiang & Gorman, 1983. = Pgm2-a,Pgm2-b (Table 1).
Pgm2-a,Pgm2-b	Table 1.

Sod,sod	Table 1.
Spl-a,Spl-b,Spl-an,spl	Table 1.
Ti-1,Ti-2,Ti-3	Singh et al., 1969; Hymowitz & Hadley, 1972. = Ti-a,Ti-b,Ti-c (Table 1).
Ti-a,Ti-b,Ti-c,ti	Table 1.
To-3,to-3	Gorman & Kiang, 1978. = Sod,sod (Table 1).
To4,to4-n	Kiang & Gorman, 1983. = Sod,sod (Table 1).

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V. RESEARCH NOTES

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1) Flecked pigmentation of soybean seed coats.

Genetic type T85 has black seed coats that are flecked with brown pigmentation. Flecking has been reported to be controlled by a single dominant gene, *Fl* (Woodworth in Morse and Cartter, 1937). This gene has been transferred into 'Clark' by backcrossing (L73-1004; R. L. Bernard, personal communication). Patterned seed coats have been observed in *Glycine soja* and *G. gracilis* (Stelly and Palmer, 1979).

Observed segregations (Table 1) from the cross of 'Blackhawk' x PI 65388 indicated the presence of a single dominant gene for brown-flecked black seeds vs. buff seeds. Blackhawk has gray pubescence (t), white flowers (w_1) and buff hila, whereas PI 65388 is a *gracilis*-type soybean with brown pubescence (T), purple flowers (W_1) and self-brown seed (probably r). Two lines were chosen from Blackhawk x PI 65388 for further study; these were:

OX312 Buff seed coat (t i W_1)
OX316 Flecked brown-black seed (t i W_1).

OX316 was crossed to T85 and L73-1004 for an allelism test.

OX 316 x T85. Of 56 F_2 plants classified, all were flecked brown-black.

OX 316 x L73-1004. Seed on F_1 plants was flecked brown-black. Of 144 F_2 plants classified, all had flecked brown-black seed, thereby confirming that the gene from PI 65388 is *Fl*. The 108 brown-pubescent plants had intensely pigmented black seed coats with brown flecks, whereas the 36 gray-pubescent plants had less-intensely pigmented black seed coats with brown flecks. In the presence of T it was necessary to classify high-moisture seeds because, in some seeds with dry-down, the black pigment coalesced sufficiently to obscure the flecking pattern.

OX312 and OX316 were test-crossed to OX379 (t i w_1), a line which should carry R, since it was obtained as an i-mutation from 0-201855, a gray-hilum line (I R).

Table 1. Segregation for patterned black pigmentation in material having purple flowers (W_1) and gray pubescence (t); from Blackhawk x PI 65388

Progenies	Seed-coat color	No.
F_6		
Nonsegregating	Flecked brown-black	8
Segregating	Flecked brown-black/buff	22
Nonsegregating	Buff	6
Chi-square for 1:2:1 ratio = 2.000		
P = 0.50-0.30		
F_7 of the 22 segregating F_6		
	Flecked brown-black	394
	Buff	119
Chi-square for 3:1 ratio = 0.796		
P = 0.40-0.30		

<u>OX379 x OX316</u> (results from high-moisture seeds)

		No. of F_2 plants
Purple flower	Flecked brown-black seed	69
Purple flower	Imperfect-black seed	24
White flower	Flecked buff seed	15
White flower	Buff seed	9

Based on OX316 carrying *Fl* and OX379 *fl*, these results give a good fit (P = 0.50-0.30) to an expected ratio of 9 (W_1 *Fl*): 3 (W_1 *fl*): 3 (w_1 *Fl*): 1 (w_1 *fl*). Thus, the W_1 gene, which is involved in the formation of delphinidin (Buzzell and Buttery, 1982), affects the black pigmentation but is not needed for the expression of the *Fl* gene. However, the buff seed must be classified before the seed dries down at maturity in order to observe the flecking pattern accurately.

OX379 x OX312 (results from dry seeds)

		No. of F_2 plants
Purple flower	Flecked brown-black seed	61
Purple flower	Imperfect black seed	20
Purple flower	Buff seed	26
White flower	Buff seed	27

Based on OX379 being *fl R* and OX312 being *Fl r*, the testcross results give a good fit (P = 0.70-0.50) to an expected ratio of 27 (W_1 *R Fl*):9(W_1 *R fl*): 12 ($W_1 r$ -):16(w_1 --). Thus, both *R* and *Fl* affect flecked seed coat and the segregations observed in Table 1 are for *R/r* and not for *Fl/fl*. Gene *R* is needed for the development of black pigments (Bernard and Weiss, 1973), and therefore, is needed for the expression of *Fl* in flecked brown-black seed.

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1) Soybean linkage group 1 tests.

Linkage group 1 is known to contain *E1*, *Df5*, *Fg3*, *Fg4*, *T* and *Y12*. *Df5* and *T* are linked with about 14.5% recombination (Palmer, 1984). Results reported by Buzzell (1978, 1979) indicated that *Rmd* from 'Altona' and 'Blackhawk' might be loosely linked to *Fg3* and *T*. Additional results (Table 1) using T233, a mutant from 'Hawkeye' (a sister line of Blackhawk), indicate that *Rmd* is not linked to *Fg3*, *T* and *Y12*.

Table 1. Soybean F_2 linkage tests[+]

Genes	a	b	c	d	Sum	R%	S.E.	Phase[++]
			Ross (*rmd fg3 Y12 T*) x T233 (*Rmd Fg3 y12 t*)					
Rmd rmd T t	397	112	122	42	673	52.8	2.8	R
Rmd rmd Y12 y12	391	119	125	39	674	50.3	2.9	R
Rmd rmd Fg3 fg3	72	19	25	4	120	I	--	C
Y12 y12 Fg3 fg3	297	136	136	0	569	0.0	--	R
Y12 y12 T t	743	112	104	165	1124	21.8	1.4	C
Fg3 fg3 T t	289	145	131	5	570	19.9	4.0	R
			L67-2234 (*E1 T Y12*) x T233 (*e1 t y12*)					
Y12 y12 T t	202	34	21	48	305	19.7	2.6	C
Y12 y12 E1 e1	201	35	16	53	305	17.1	2.4	C
E1 e1 T t	215	2	8	80	305	2.6	0.1	C
			OX 937 (*fg4 t*) x Chippewa 64 (*Fg4 T*)					
Fg4 fg4 T t	342	8	10	108	468	3.9	0.9	C

[+]Product method, Immer and Henderson (1943).

[++]R = repulsion; C = coupling.

Results in Table 1 indicate a close linkage between *Fg3* and *y12*. The *fg3 y12* recombinant has been obtained; additional test results are being obtained to determine the recombination between *Df5 Fg3 T Y12* (Buzzell and Palmer, unpublished).

Buzzell and Walker (1982) reported obtaining the *fg4 t* recombinant. A linkage test of the *Fg4* and *T* genes indicates 3.9% recombination (Table 1).

Forty-three backcross-derived isolines for various genes were obtained from R. L. Bernard, University of Illinois. The flavonol class of these isolines was determined; one isoline, L63-1097, was different from the Harosoy recurrent parent. L63-1097 is 4t (*fg1 Fg2 Fg3*), whereas Harosoy is either 6t (*fg1 Fg2 fg3*) or 2t (*Fg1 Fg2 fg3*), depending on the line. L63-1097 carries *pc* (curly pubescence) from T141, which is *fg1 Fg2 Fg3*. The occurrence of *Fg3* with *pc* after five backcrosses suggests that *pc* and *Fg3* may be closely linked. However, the *pc* curly pubescence isoline of Clark, L63-2435, is 6T like Clark and, thus, does not carry *Fg3* from T141. The linkage group for *pc* is not known; Keaschall et al. (1981) have shown that it is not linked to *w1* in linkage group 8. A linkage test is being run to determine whether or not *pc* is in linkage group 1.

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1) Environmental impact on different characteristics of soybean.

The leguminous protein and oil rich crop soybean requires specific environment for its successful growth and yield. The variation in yield levels and restricted adaptation are mostly due to photoperiodic requirements (Weber and Moorthy, 1952) and probably due to thermal sensitivity (Leng, 1968). The nature and magnitude of variability present in an individual or a population is due to both genetic and nongenetic causes. The environment itself represents the nongenetic influence for the expression of different characteristics of an individual. Thus, to identify the suitable genotypes and the proper sowing time, it is of utmost necessity to grow a number of genotypes in a series of environments.

Soybean is still a new commercial crop in northeastern India and a number of exotic genotypes have been introduced from different places. Hence, the present investigation has been designed to identify the suitable genotypes and sowing time for these genotypes.

Materials and methods. The investigation comprised ten soybean genotypes. Bragg, JS 2, PK 369, Kalitur, JS 72-1, PK 71-21, PK 327, JS 72-375, PK 409, and DS 73-16 were grown in 6 environments: 3 in the spring and 3 in the summer season, and the environments were the sowing dates, viz., E1 (28.12.81), E2 (28.1.82), E3 (3.3.82), E4 (17.7.82), E5 (18.8.82) and E6 (20.9.82).

The whole experiment was carried out in the Instructional cum Research Farm of the Department of Plant Breeding and Genetics, Assam Agricultural University, Jorhat, Assam, India, with 3 replications in each environment. The genotypes were alloted randomly in the 10 plots of each replication. Five rows were made in each plot measuring 2 m length, the plant-to-plant distance was 10 cm and row-to-row 45 cm. Observations were recorded from 10 randomly selected plants of each plot, leaving the border rows and plants on yield and other morphophysiological characters. The data were analyzed in each environment to determine the environmental mean; the environmental indices were analyzed following the method given by Eberhart and Russell (1966).

Results and discussion. The environmental mean, i.e., the mean of all the genotypes at a particular environment, and the environmental index, i.e., the difference between the environmental mean and grand mean of the genotypes over all the environments (Eberhart and Russell, 1966), and the genotypes permit the assessment of the best and the poorest performing environments for various characters.

Out of the six environments, E3 (3.3.82) was observed to cause decided improvement in number of primary branches, pod length, plant height at 50% flowering, and the seed yield per plant as inferred by the environmental mean and indices. The environment E2 was also found to be superior due to the high environmental mean and index for number of secondary branches, number of pods per plant, number of pods per cluster, number of seeds per pod, and days from flowering to maturity. The E1 (28.12.81) also exhibited high environmental mean and index for number of pods per plant and seed yield per plant. This environment also caused decided improvement in number of clusters per plant. However, the environment was observed to cause delayed flowering and maturity, as indicated by the highest environmental mean and environmental index for days to flowering and days to maturity. For days to flowering and maturity, the minimum duration was expressed in E6 (20.9.82), which was in the summer season. For spring season E3 (3.3.82) followed by E2 (28.1.82) exhibited moderate duration for flowering to maturity. All the results were suggestive of better environmental condition for soybean growing during spring rather than summer season. It appeared that sowing in between the last week of January and first week of March provides the most ideal environment for the best performance of soybean crop with an optimum crop duration. However, it is necessary to conduct multilocation trial involving sowing during spring season in order to identify and recommend the suitable sowing time of soybean in northeast India.

In this investigation, the genotypes were observed to possess a tendency to have more or less reduced flowering and maturity duration from E1 to E6. This might be due to the combined influence of temperature, humidity, and day length on flowering and maturity duration of the crop.

Summary. Spring sowing of soybean was found to be more congenial than that of summer sowing, which is the normal cultivation season in northeastern India. It appeared that sowing between last week of January and first week

Table 1. Environmental mean (m) and environmental index (I) for the characters under study in all environments

Environmental mean (m) and Index (I)	Environments E1	E2	E3	E4	E5	E6
Days to flowering						
m	60.50	48.64	48.88	35.54	32.86	27.78
I	18.13	6.27	6.51	-6.83	-9.51	-14.59
Plant height at 50% flowering (cm)						
m	25.10	34.58	42.10	48.50	21.04	13.81
I	-5.75	3.73	11.25	17.65	-9.81	-17.04
Number of primary branches						
m	4.80	4.63	4.89	3.93	2.00	2.13
I	1.07	0.90	1.16	0.20	-1.73	-1.60
Number of secondary branches						
m	1.27	1.86	1.62	0.76	0.62	0.87
I	0.10	0.69	0.45	-0.41	-0.55	-0.30
Number of pods per plant						
m	58.83	59.01	43.14	33.03	11.33	9.78
I	22.98	23.16	7.29	-2.82	-24.52	-26.07
Number of clusters per plant						
m	19.97	18.94	11.63	10.98	4.32	3.57
I	8.40	7.37	-0.06	-0.59	-7.26	-8.00
Number of pods per cluster						
m	3.00	3.34	2.20	3.23	2.82	2.76
I	0.11	0.45	-0.69	0.34	-0.07	-0.13
Number of seeds per pod						
m	2.93	3.20	2.83	2.44	2.42	2.42
I	0.22	0.49	0.12	-0.29	-0.29	-0.29
Pod length (cm)						
m	3.97	3.99	4.51	3.44	2.93	2.76
I	0.37	0.39	0.91	-0.16	-0.67	-0.84
Leaf area (sq cm)						
m	10.36	16.03	35.43	41.34	21.66	16.25
I	-13.15	-7.48	11.92	17.83	-1.85	-7.26

Table 1. Continued

Environmental mean (m) and Index (I)	Environments					
	E1	E2	E3	E4	E5	E6
Days of maturity						
m	104.84	95.76	97.36	97.41	91.63	85.63
I	9.41	0.33	1.93	1.98	-3.80	-9.80
Days from flowering to maturity						
m	56.67	65.69	48.00	64.26	51.40	52.12
I	0.31	9.33	-8.36	7.90	-4.96	-4.24
Plant height at 50% maturity (cm)						
m	29.86	44.08	67.84	56.85	21.38	13.87
I	-9.12	5.10	28.86	17.87	-17.60	-25.11
100-seed weight (g)						
m	13.75	11.50	13.95	14.63	13.31	12.54
I	0.47	-1.78	0.67	1.35	0.03	-0.74
Seed yield per plant (g)						
m	10.86	8.32	11.85	8.38	4.60	5.28
I	2.64	0.10	3.63	0.16	-3.62	-2.94

of March provides the most suitable environment for the better performance of soybean with an optimum crop duration. However, multilocational trial is necessary to determine and recommend the spring sowing of soybean in this region of India.

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1) Intra-plant variation in mutation frequency and spectrum in soybean.

Upadhyaya (1976) observed that the number of mutant plants was exceedingly low in segregating M2 progenies in soybean. In M3 progenies of normal M2 plants, the number of segregating progenies was also not very high as compared with nonsegregating progenies. But, all the segregating progenies in M3 generation showed an excellent fit to the 3 normal:1 mutant ratio, indicating mutant as a monogenic recessive trait. Such a situation was encountered in many cases of albino, yellow leaf, crinkled leaf, and sterile mutants.

In most of the sexually propagated crops with short life cycle, like soybean, mutation induction for plant breeding purposes is preferably done by seed treatment. It is essentially a treatment of embryo meristems. In such crops, diplontic selection may result in the differences with respect to the frequency of mutated sectors in different plant parts or branches. Further, there may be more than one initial cell for the formation of branches and any of them may be mutated. Therefore, it was considered desirable to study the differences, if any, between different primary branches of each M1 plant with respect to mutation frequency and spectrum.

Materials and methods. Samples of 200 seeds of Bragg and Type-49 soybeans were irradiated with 15 krad of gamma-rays. Irradiated seeds along with controls were planted and observed throughout the season. The branches of each M1 plant in both the varieties were threshed separately and kept in separate packets. Progeny rows of each branch were sown and mutations were recorded for different qualitative characters. Observations on pollen fertility were recorded. The lines segregating for sterility could be easily identified at maturity, since the sterile plants remained green, whereas the normal ones matured normally. The ratios of normal versus mutants were determined for each of the segregating branch progenies. Chi-square test was employed to test the goodness of fit of genetic ratios.

Results and discussion. Four types of qualitative mutants (i.e., albino or yellow leaf, curved leaf, crinkled leaf, and sterile) were observed in M2 generation. The different branches of each of the M1 plants showed the differential behavior in releasing the mutant loci. In Bragg 15 krad a total of 82 branches out of 298 planted showed the mutations for leaf color (albino or yellow), leaf shape (curved or crinkled) and sterility. Of the total, sterile mutants were most frequent (79.27%) followed by chlorophyll deficient (12.34%), crinkled (6.17%) and curved leaves, which were lowest in frequency (2.43%). Of the branches mutating, first branch showed the maximum mutation (31.70%), followed by second branch (23.17%), third branch (19.51%) and fourth branch (14.63%). The seventh branch showed the lowest frequency of mutations of only 2.43% and that too only sterile mutants could be recovered (Table 1).

In the first branch, sterile mutants were most frequent and about 28.05% of sterile mutants were recovered as compared to crinkled leaf (2.43%) and chlorophyll deficient mutants (1.21%). Similarly, second branch also showed predominance of sterile mutants (15.85%) followed by 4.87% chlorophyll deficient mutants and 1.21% each curved and crinkled leaf mutants. The sixth and seventh branches showed only sterile mutants, 3.65 and 2.43%, respectively, and no mutants for leaf deformities or chlorophyll deficiency in Bragg.

In Type-49, the pattern was more or less similar and 27.12% of the branches showed segregation for different types of mutants. Out of a total 83 lines segregating, 80.72% were segregating for sterile mutants. The branchwise breakup of this was 22.89% in first, 12.05% in second, 13.25% in third, 12.05% in fourth, 8.43% in fifth, 6.02% in sixth, 3.61% in seventh, and 1.20% each for eighth and ninth branches. The mutants for leaf deformities such as crinkled and curved leaves were observed in first (2.40%) and second (1.20%) branches only. Albino and yellow leaf mutants were found in the progenies of first to fifth branches with relative frequencies of 3.61, 4.82, 2.40, 1.20 and 3.61%, respectively. Overall frequency of all types of mutants was highest for first branch (28.92%) followed by second (18.02%) and third (15.66%) branches. Eighth and ninth branches again showed lower number of mutants with a frequency of 1.20% each. The inheritance pattern of these mutants was studied in M2 generation and confirmed in M3 generation. All of them were monogenic recessive and a good fit to 3 normal:1 mutant segregation with high probability was observed.

Table 1. Branchwise frequency of different qualitative mutants in M1 plants of Bragg and Type-49 varieties of soybean induced by 15 krad of gamma-rays

Variety	Segregating for	Number of branches I	II	III	IV	V	VI	VII	VIII	IV	Total frequency
Bragg	Yellow leaf and albino	1 (1.21)+	4 (4.87)	2 (2.43)	2 (2.43)	1 (1.21)	--	--	--	--	10 (12.34)
	Curved and crinkled leaf	2 (2.42)	2 (2.42)	1 (1.21)	2 (2.42)	--	--	--	--	--	7 (8.60)
	Sterility	23 (28.05)	13 (15.85)	13 (15.85)	8 (9.76)	3 (3.65)	3 (3.65)	2 (2.42)	--	--	65 (79.27) 82‡ (27.52)
Type-49	Yellow leaf and albino	3 (3.61)	4 (4.82)	2 (2.40)	1 (1.20)	3 (3.61)	--	--	--	--	13 (15.66)
	Curved and crinkled leaf	2 (2.40)	1 (1.20)	--	--	--	--	--	--	--	3 (3.60)
	Sterility	19 (22.89)	10 (12.05)	11 (13.25)	10 (12.05)	7 (8.43)	5 (6.02)	3 (3.61)	1 (1.20)	1 (1.20)	67 (80.72)
	Total	50 (30.30)	34 (20.61)	29 (17.58)	23 (13.94)	14 (8.48)	8 (4.85)	5 (3.03)	1 (0.61)	1 (0.61)	83‡ (27.12)

+Values in parentheses are percentages.

‡Total frequency of mutants was tabulated over total number of lines planted (298 for Bragg and 306 for Type-49).

In this investigation, the results clearly revealed that mutations are generally produced in one or more early branches and the later formed branches seldom had mutations. A close perusal of the data reveals that the first two or three branches showed higher mutation frequency while the rest of the branches were completely normal. Thus, if an M1 plant is threshed as a whole and M2 progeny rows are raised from the bulk seed, then in the M2 generation the number of mutant plants is expected to decrease considerably and there may not be a good fit to the 3 normal:1 mutant ratio, as observed by Upadhyaya (1976). Subsequently, in the M3 generation, the expected ratio of 2 segregating:1 nonsegregating lines out of normal M2 plants sown may not be observed and results may not be in the expected pattern based on a particular hypothesis. Therefore, if M2 generation is raised by bulking the seeds of each M1 plant, the conclusions regarding genetic ratio should be delayed till the segregation pattern in M3 progenies is studied.

This situation seems to be analogous with ones observed in barley (Gaul, 1961), wheat (Goud, 1967) and rice (Yamaguchi, 1962). In wheat, there are five or six ear initials and if one of the initials is affected, we get the mutant individuals in the M2 population (Frydenberg, 1963). Further, Gaul (1916) reported that tillers in barley that developed later during the ontogeny of M1 plants showed a lower mutation frequency than those that developed early. In our investigation, we also observed similar pattern in soybean, except that branches substitute for tillers of cereals. The early-developed branches showed maximum mutation frequency and the late ones the lowest frequency of mutations.

In mutation breeding experiments, the sampling of branches of M1 plants is, therefore, recommended rather than the M2 seeds to obtain wide range of mutations and for employment of effective selection program subsequently. As is evident from the results of this study, the first four branches show above 80% of total mutations frequency (Table 2); therefore, while sampling these should be invariably included. In wheat, Goud (1967) has suggested the separation of first formed five or six tillers to recover higher mutation frequency.

Regarding the origin of branches in soybean, two possibilities are suggested on the basis of results obtained in the present study: (i) there may be two initial cells which may give rise to the alternate branches, or (ii) there may be different initials for different branches. Thus, any of the

Table 2. Total mutation frequency of different qualitative mutants in soybean

Variety	First two branches	Firt three branches	First four branches
Bragg	54.82	74.31	88.93
Type-49	46.97	62.62	75.87
Average	50.90	68.47	82.40

initials may be affected by the mutagenic treatment and the chances of simultaneous mutations affecting the same locus in all the initials are extremely rare. The latter possibility seems to be more likely. Existing literature also supports the hypothesis of existence of the different initials for different branches as in pea (Blixt et al., 1958). Monti (1965) concluded that five was the highest number of cell initials in an apical meristem of a primary stem and four for a secondary stem.

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2) Induced variability for quantitative characters.

Gamma rays were used to induce the genetic variability for different quantitative characters in Bragg and Type-49 varieties of soybean.

Materials and methods. Samples of 200 seeds of Bragg and Type-49 soybeans were irradiated with 10 krad, 15 krad and 20 krad doses of gamma rays, a week before planting time. These irradiated seeds, along with the unirradiated controls, were planted in split-plot design with three replications. Eight seeds from each plant of M1 generation were taken and treatment-wise bulks were prepared. Planting was done in split-plot design using Bragg and Type-49 as main plots and doses as sub plots with two replications. Each plot consisted of 5 rows 6 m long and 60 cm apart. Twenty-five plants were tagged at about 15 days after germination to record the observations on different quantitative characters. Analysis of variance was conducted and, in order to test whether treated populations had significantly more variability than their respective controls in the M2 generation, the "F" test was used. It was assumed that the control populations could provide an estimate of environmental variability and the treated populations would include environmental as well as induced genetic variability. Heritability in broad sense was estimated only in those populations that had significant increase in variance over control.

Results and discussion

Days to flowering. Type-49 took more days to flower than Bragg (Table 1). The irradiated populations of Bragg and Type-49 did not differ significantly

from their respective controls in respect to mean for this character. The extent of variability in the irradiated populations of both varieties was more than their control populations, but differences were significant only in the case of Type-49 15 krad and 20 krad with heritability of 65.5% and 65.6%, respectively.

Days to maturity. Varieties differed significantly with respect to number of days taken to maturity. Bragg matured earlier than Type-49. The effects of doses were significant only in Bragg. In case of Bragg 10 krad as well as Bragg 15 krad, the maturity was delayed by 4 and 3 days, respectively (Table 1). The estimated variance of the different treated populations was consistently higher than their respective controls; however, significant increase was observed only in case of Type-49 10 krad, 15 krad and 20 krad populations. None of the populations of Bragg that showed delayed maturity could show any significant increase in variance, indicating very little or no chance of selection. In Type-49 10 krad, 15 krad, and 20 krad populations, the broad sense heritability estimates were 51.8, 55.1 and 69.1%, respectively.

Plant height. Both the varieties differed significantly from each other with respect to plant height. Type-49 was taller than Bragg (Table 1). The overall effect of 10 krad was to shorten the plant height in both the varieties. However, differences were nonsignificant in the case of Bragg. Treatments with 10 krad and 15 krad doses of gamma rays decreased the plant height of Type-49 significantly.

Even though the average plant height of treated Bragg populations did not differ significantly from their control, the increase in the estimated intrapopulation variance was observed for 10 and 20 krad doses as compared to 0 krad. The broad sense heritability estimates were 38.1 and 41.9%, respectively, in Bragg 10 krad and 20 krad populations.

Primary branches per plant. The effect of different doses of gamma-rays was insignificant and the increasing or decreasing effects were not consistent. The intrapopulation variance of treated populations was not significantly different than the control, indicating virtually no mutations for the genes controlling this character (Table 1).

Pods per plant. Only the dose of 20 krad in Bragg (69.7 pods/plant) induced significantly higher number of pods per plant as compared to Bragg 0 krad (50.3 pods/plant). Highly increased intrapopulation variances were observed only in Bragg 10 krad, 15 krad, and 20 krad populations, as compared with Bragg

Table 1. Extent of variability for different quantitative characters in irradiated populations of soybean

	Bragg				Type-49				
Characters	0 krad	10 krad	15 krad	20 krad	0 krad	10 krad	15 krad	20 krad	C.D.
Days to Flowering									
Mean	40	40	39	40	61	61	62	62	1.3
Variance	0.88	0.94	1.12	1.21	1.01	1.25	2.93**	3.02**	--
Heritability (b)	--	--	--	--	--	--	65.5	66.6	--
Days to Maturity									
Mean	109	113*	112*	110	120	118	120	121	2.3
Variance	2.01	2.00	2.85	2.83	0.93	1.93**	2.07*	3.01*	--
Heritability (b)	--	--	--	--	--	51.8	55.1	69.1	--
Plant Height									
Mean	42.7	38.5	47.5	37.1	110.0	102.5*	103.6*	112.3	9.2
Variance	38.1	61.6*	57.3	65.6*	382.8	468.1	537.3	596.1	--
Heritability (b)	--	38.1	--	41.9	--	--	--	--	--
Branches per plant									
Mean	4.3	5.4	5.7	5.3	5.6	5.3	6.0	5.8	1.6
Variance	2.9	3.3	2.5	3.9	3.6	4.6	3.7	4.5	--
Heritability (b)	--	--	--	--	--	--	--	--	--
Pods per plant									
Mean	50.3	65.1	53.1	69.7*	68.3	79.2	68.9	64.8	18.7
Variance	400.9	647.3*	838.8**	781.4**	2043.0	2532.8	1923.8	2067.0	--
Heritability (b)	--	38.1	52.2	48.7	--	--	--	--	--
Seeds per pod									
Mean	2.10	2.21*	2.07	2.11	1.99	1.93	1.86*	1.86	0.07
Variance	0.04	0.06	0.06	0.06	0.05	0.06	0.08*	0.09*	--
Heritability (b)	--	--	--	--	--	--	37.5	44.4	--

continued

Table 1. Continued

Characters	Bragg				Type-49				C.D.
	0 krad	10 krad	15 krad	20 krad	0 krad	10 krad	15 krad	20 krad	
100-seed Weight									
Mean	15.1	15.3	17.2	16.5	9.7	10.3	10.7	10.7	2.3
Variance	6.4	5.3	26.6**	10.5*	1.7	1.6	2.7*	3.4**	--
Heritability (b)	--	--	75.8	38.8	--	--	37.3	50.0	--
Yield per plant									
Mean	15.9	20.0	16.8	20.1	11.4	11.3	11.5	11.7	4.6
Variance	59.1	82.6	101.1*	91.7	56.1	45.3	63.1	108.0*	--
Heritability (b)	--	--	41.5	--	--	--	--	48.1	--

*Significant at 5 percent level of significance.

**Significant at 1 percent level of significance.

control. The broad sense heritability estimates were 38.1% for Bragg 10 krad, 52.7% for Bragg 15 krad, and 48.7% for Bragg 20 krad (Table 1). The increase in mean number of pods per plant of Bragg 20 krad accompanied by higher estimates of variability and heritability suggests definite possibility of effective selection in this population.

Seeds per pod. The different doses of gamma rays had decreasing effect on seeds per pod in Type-49, but the number of seeds per pod was significantly higher in Bragg 10 krad only as compared with Bragg control. The estimates of intrapopulation variances were significantly greater than their control only in Type-49 15 krad and Type-49 20 krad, with broad sense heritability of 37.5 and 44.4%, respectively (Table 1).

100-seed weight. Bragg had bold seeds (16 g per 100 seeds) as compared to Type-49, which had 100-seed weight of 10 g. The mean weight of 100 seeds of different treated populations did not differ significantly from their controls, but the presence of higher intrapopulation variances in irradiated populations was observed in 15 krad and 20 krad treatments of both the varieties. In Bragg 15 krad, the variance was 26.6 as against 6.4 of Bragg 0 krad (Table 1). The range of 100-seed weight in the irradiated populations was very high and in Bragg it was as low as 10 g per 100 seeds to as high as 24 g, justifying the higher variance accompanied by no difference in mean seed weight of population as such. Heritability estimates in broad sense were 75.8% for Bragg 15 krad, 38.8% for Bragg 20 krad, and 50.0% for Type-49 20 krad, indicating that plants with bold and small seeds can be selected.

Yield per plant. Type-49 was poor yielder as compared with Bragg, which, due to its high yielding ability, is used as check variety in soybean experiments at country level. When averaged over both the varieties, treatment with gamma rays did bring some increasing effect, but these differences were not statistically significant. Bragg 10 krad and Bragg 20 krad populations had per plant yield of 20 g each as against 16 g of Bragg 0 krad (Table 1). Within-population variances of the irradiated populations were higher in most of the cases, but significant differences were observed only in case of Bragg 15 krad and Type-49 20 krad, with 41.5% and 48.1% broad sense heritabilities, respectively.

Significant increase in variances of treated populations over controls for most of the quantitative characters and fairly high heritability for yield components offer a definite possibility of improving these characters by

further selection. In most of the cases, the mean of the treated populations was either slightly better or similar to the untreated control, which indicated that micro mutations were positive as well as negative. In this investigation, the relative superiority of the mean of some of the treated populations further indicate that positive micro mutants were more frequent than the negative ones. Our earlier studies (Upadhyaya and Singh, 1979) also indicated the increased variance of treated populations. For number of primary branches per plant, the intrapopulation variance of treated populations was not significantly higher than the control. In our earlier studies (Upadhyaya and Singh, 1979) also, this character showed similar behavior. The possible reason for such behavior may be either the number of genes responsible for branches is quite little or they are quite resistant for mutagens as compared with other characters.

The dose of 20 krad gamma rays was found to be most effective in inducing genetic variability for yield and contributing characters.

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3) A narrow leaf type soybean variety - PK-308

'PK-308' has been developed at this station from a cross of T-31 x Hardee following the pedigree method of breeding. It has been released by the Central Varietal Release Committee, Government of India, in 1984, for general cultivation in the northern plains of India. This is the first narrow-leaf type cultivar released in this country. It has outyielded 'Bragg' on an average by 16.75% over 5 years of coordinated testing in the northern plains (Table 1). In the Soybean Preliminary Observation Trials (SPOT) of INTSOY, 1982, PK-308 yielded 2240 kg/ha and occupied ninth rank in a trial of 16 varieties.

Being a narrow leaf type variety, PK-308 is expected to be superior to Bragg (broad leaflet) under intercropping and this has been demonstrated in intercropping experiment with maize at Pantnagar, where PK-308 has outyielded Bragg by 29.27% (Table 2).

Table 1. Yield (kg/ha) of PK-308 in the northern plain zone

Variety	Delhi	Pantnagar	Ranchi	Kalyani	Behrampur	Mauranipur	Mean
			1978				
Bragg	1260	2820	2806	757	1961	1040	1774
PK-308	1382	2959	2194	939	1993	1064	1755
CD 5%	213	715	356	267	249	--	
CV %	11	20	11	26	8	--	

1979

	Delhi	Pantnagar	Ranchi	Hissar	Mean
Bragg	1561	1328	1792	2177	1715
PK-308	1354	1701	1667	2929	1913
CD 5%	132	560	389	--	
CV %	6	31	14	--	

1980

	Delhi	Pantnagar	Ranchi	Mean
Bragg	2106	1342	1939	1796
PK-308	1917	1817	2006	1913
CD 5%	223	363	403	
CV %	21	15	14	

1981

	Delhi	Pantnagar	Ranchi	Mean
Bragg	1141	1343	1590	1358
PK-308	1024	1929	2378	1777
CD 5%	273	297	186	
CV %	13	16	8	

1982

	Pantnagar	Haldwani	Jorhat	Mean
Bragg	2153	2178	2786	2372
PK-308	2283	2600	4618	3167

Overall average

Bragg	1803
PK-308	2105 (16.75% increase over Bragg)

NB:CD and CV rounded off to full figures.

Table 2. Yield of PK-308 (kg/ha) in comparison with Bragg under intercropping with maize at Pantnagar in 1983*

Treatment	Grain yield (kg/ha)	
	Maize	Soybean
Pure maize at 60 cm row spacing	3697	--
Paired row of maize at 30/90 cm spacing	3488	--
Maize at 60 cm + 1 row of soybean in between 2 rows of maize (Bragg)	3488	854
Maize at 60 cm + 1 row of soybean in between 2 rows of maize (PK-308)	3502	1104
Maize at 60 cm + 1 row of soybean in between 2 rows maize (Shilajeet)	3697	1095
Paired rows of maize 30/90 cm + 2 rows of soybean (Bragg)	3488	898
Paired rows of maize 30/90 cm + 2 rows of soybean (PK-308)	3605	1161
Paired rows of maize 30/90 cm + 2 rows of soybean (Shilajeet)	3627	870
Sole crop of soybean (Bragg)	--	1984
Sole crop of soybean (PK-308)	--	2532
Sole crop of soybean (Shilajeet)		2243
Pure maize at 60 cm (Dummy treatment)	3746	--
SEM	0.692	0.875
CD 5%	2.08	2.620
CV %		10.71

Yield superiority of PK-308 over Bragg (Treatment 3 vs. 4) 29.27%.
Yield superiority of PK-308 over Bragg (Treatment 6 vs. 7) 29.28%.

*Data taken from project coordinator's report and summary tables of experiments, 1983-84, G.B.P.U.A.T., Pantnagar.

PK-308 is moderately resistant to yellow mosaic (yellow spots remain small) as compared to Bragg, which is susceptible and suffers severely in the northern plains. PK-308 is resistant to bacterial pustules (*Xanthomonas phaseoli* var. *sojensis*) and Alternaria leaf spot.

PK-308 has about 20% oil and 40% protein (Table 3). It has grey pubescence and white flowers. Plant height is 50-65 cm. It matures in about 110 days and is a week earlier than Bragg. Seeds are attractive yellow and medium in size (12 g/100 seed).

Table 3. Oil and protein content in PK-308

	1981		1982		Mean	
	Oil %	Protein %	Oil %	Protein %	Oil %	Protein %
Bragg	20.94	40.05	21.61	39.73	21.27	39.89
PK-308	20.52	38.11	19.94	42.53	20.23	40.32

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1) Retention of impermeability and viability of soybean seeds under water submergence

Hard and impermeable seeds in soybean, as in other grain legumes, are of common occurrence. Various workers have studied this trait in soybean and attributed it to both genetical (Woodworth, 1933; Green and Pinnell, 1968; Kilen and Hartwig, 1978; Srinives, 1980; Srinives and Hadley, 1980; Rana et al., 1981; Shahi and Pandey, 1982) and environmental (Archavaleta and Snyder, 1981; Gupta et al., 1981, 1982) factors. Shahi and Pandey (1981) found association of hard seeds with lateness in flowering and maturity. Maxey and Delouche (1980) and Minor and Paschal (1982) reported association of hard seeds and longer storage life. Rana et al. (1982) found positive association of hard seeds with crushing hardness but negative correlation with water absorption, percent laboratory germination and boldness index. Kolykos (1952) found positive association of hard seeds with grain yield, but Garg (1979) and Rana et al. (1982) did not observe any such correlation. Rana (1977, unpublished) found seasonal variation in occurrence of hard seeds in popular soybean varieties like Punjab-1 and Lee. Such seeds when grown under field conditions were found to give normal germination (93%) and no hard seeds were found in the harvest obtained from them, when grown under normal conditions of soil moisture throughout the plant life. Though hard seededness or impermeability to water may not influence field germination, it does become a problem when soybean is to be cooked whole after soaking. The resultant preparation does not yield uniformly cooked beans unless over-cooked.

The investigation being reported herein was not carried out as a strictly laboratory experiment, yet efforts were made to simulate laboratory conditions at home as far as possible. The objective was to find out whether soaking the beans beyond 24 hours would help in reducing their number in a given lot to a desirable extent. The results given in Table 1 show that submergence of impermeable seeds in tap water even after a week did not reduce their number appreciably. The experiment, though not initially planned to last that long, had to be continued for 19 months in order to see the persistence of impermeability under total submergence in the material being handled.

Table 1. Number of seeds imbibing water in successive weeks out of a lot of 1000 soybean seeds containing about 50% hard seeds

Week or day no.	No. of seeds softened	Progressive total	Week no.	No. of seeds softened	Progressive total
Day			31	2	942
1	495	495	32	1	943
2	40	535	33	3	946
3	33	568	34	5	951
4	24	592	35	1	952
5	20	612	36	1	953
6	15	627	37	1	954
7	15	642	39	1	955
			42	1	956
Week			44	1	957
2	50	692	45	4	961
3	28	720	46	1	962
4	51	771	48	2	964
5	24	795	49	1	965
6	24	819	52	1	966
7	27	846	53	1	967
8	8	854	54	1	968
9	13	867	57	1	969
10	10	877	58	2	971
11	11	888	59	2	973
12	9	897	60	2	975
13	5	902	61	1	976
14	6	908	63	3	979
15	2	910	67	1	980
16	4	914	68	2	982
17	3	917	69	1	983
18	1	918	70	1	984
19	3	921	71	1	985
20	1	922	72	1	986
21	3	925	73	1	987
22	2	927	74	2	989
23	2	929	75	1	990
24	1	930	76	2	992
25	3	933	77	2	994
26	2	935	78	1	995
29	3	938	80	1	996
30	2	940	82	1	997
			83	1	998
			84	1	999
			89	1	1000

The material consisted of a mixture of rejected F_3 lines of a cross between Punjab-1 (yellow seed coat) and Himso 330 (black seed coat with green cotyledons), grown during summer, 1982. The harvest had been stored at room temperature for over six months. Among the parents, Punjab-1 is medium maturity variety and normally has no hard seeds, while the other parent is late and does contain 2-5% hard seeds. However, the mixture had all shades of seed coat color, that is, yellow, brown, black, and mottled, and both yellow and green cotyledons. However, it contained about 50% hard seeds.

One thousand seeds were picked randomly from this lot and soaked in tap water on May 9, 1983. After 24 hours of soaking at room temperature, only 495 seeds were found to have imbibed water and the rest had settled at the bottom of the bowl. The soaked seeds were removed and hard seeds were washed and again put in tap water in the same bowl. Seeds of all types of coat and cotyledon color were found in both the normal and hard seeds. During next 24 hours, only 40 seeds imbibed water and thereafter the number of permeable seeds continued declining and fell to 15 on the sixth and seventh day and to only 12 seeds on the eighth day.

On one of the days during the first week when water was not changed after removing soaked seed, both the seeds and the surface of the bowl were found to be covered with gel type stuff on the following day. Thenceforth, both the hard seeds and the container were regularly washed in running water, after removal of softened seeds. After two weeks, the seeds were transferred to a cup made of China clay and placed on the dining table lest we missed to take the count of softened seeds regularly every morning and wash the rest of them. To further facilitate the regular maintenance of record of daily observations, the number of seeds that got soaked during an interval of 24 hours was noted every morning on the wall calendar, so that any member of the family could record observations at breakfast time, whenever the author was not at home. Though observations were recorded daily as given for the first seven days in Table 1, the same have been presented in the table weekwise from the second week onward in order to shorten the length of the table.

For the first 26 weeks, at least one seed imbibed water every week, but, during the next two weeks, no seed were permeated. The table further reveals that from fifteenth week onward till the last week, the number of permeable seeds per week ranged from zero to five. During 21 out of 89 weeks, no seeds got permeated. All types of seeds, that is, those with yellow, brown, black,

and mottled seed coat or green and yellow cotyledons, continued to constitute the lot till the very end. Among the last five seeds, two had yellow, two brown and one black seed coats. Incidentally the last seed that alone resisted permeability for over a month after the whole lot had imbibed had brown seed coat and yellow cotyledons.

In order to test the viability and germinability, all seeds that imbibed water were put under moist conditions in plates or in sand beds, depending upon the situation. However, none of them was found to be inviable as all of them germinated well.

In summer 1984, five plants were raised from these hard seeds. However, the pods in two of them were eaten by rats and harvest was obtained only from three. On testing, only one of the plants yielded two hard seeds out of 38 borne on it.

Both genetic and environmental factors have been reported to be responsible for hard seededness. In the present case, one of the parents did contain some hard seeds, but the seed lot had been harvested from a crop raised under upland conditions, where the seed developed and matured under water stress. The results point to the fact that, in addition to genetic factors, the environment played a fairly great role in rendering soybean seed impermeable to water, as discussed in detail by Archavaleta and Snyder (1981).

As one of the hard seeds resisted permeability under submerged conditions for 88 weeks, while others remained impermeable for one day to 18 months, it is clear that degree of impermeability even under water submergence varied from seed to seed. Hard seeds have been reported to lose this characteristic during storage at room temperature up to one year (Shahi et al., 1982), but in the present case the impermeable seeds resisted imbibition of water even under complete submergence and thus this trait appears to have helped the genus *Glycine* in surviving the submerged conditions even for a considerably long period of time.

Conclusion that we can draw from this study is that soaking the soybean beyond one day does not reduce the hard seeds present in a lot appreciably and environment also plays an important role in the development of hard seeds. Such seeds are wholly viable and, depending upon the degree of impermeability, can retain their viability under submerged conditions for months on end. This trait is unsuitable from cooking point of view, but appears to be a desirable trait from the point of view of survival in nature and might have played an important role at different evolutionary stages of the crop.

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1) Analysis of variation and relationship between soybean traits in F_3 and F4 in two cross combinations.

Qualities and traits of soybean are reported to produce a considerable variation under conditions of long-day environment (Jaranowski et al., 1980, 1983). An outline of genetic bases for soybean breeding in a latitude above 50°N calls for establishing the least environment-modified traits and relationships.

Materials and methods: This study carries a presentation of relationships and heritability of traits in early hybrid generations (F_3-F_4) from two cross combinations, i.e., a medium-early semi-determinate line (PI 238920) and a somewhat later maturity semi-determinate line (PI 180517) -- cross combination "A", in addition to a Japanese cultivar Oyachi No. 2, classified in our conditions to late semi-determinate lines -- cross combination "B".

A characteristic of parental forms from three-year field experiments is shown in Table 1.

Table 1. Values for parental forms of soybean

Traits	PI 238920	PI 180517	Oyachi No. 2
Days to maturity	135.0	143.0	164.0
Height, cm	58.0	68.0	61.0
Branch, number per plant	4.0	4.0	2.0
Seeds, number per plant	39.0	62.0	24.0
Seed weight per plant, g	7.2	11.2	6.3
Seeds, number per pod	1.5	1.6	1.6
Seed weight, g/100	18.9	17.7	21.4

For each cross combination, a separate experiment was carried out according to the randomized block design, in four replications. Plants selected for testing were characteristic of high selective value in the F_2. From the first cross combination, 25 progenies of the F_3 were grown in the first year

of experiments and 33 of the F_4 in the consecutive year. From the second cross combination, 67 progenies of the F_3 were grown. In the consecutive year, the number of progenies were reduced to 32. Plants were grown in rows spaced 0.5 x 0.2 m. For biometric analysis, 10 plants were analyzed from each plot.

Results: Relationship estimates for developmental and morphological traits in hybrid generations are likely to help determine effective criteria for selection. In the first year, the variation of morphological traits and its effect on seed yield were analyzed. The second year included the relationship among earliness, morphological traits, and yield parameters. In the F_3 and F_4 of the two cross combinations, the values for variation coefficients were relatively low in the two years of experiments. The lowest variation was noted for the number of seeds per pod (Table 2).

A relatively high genotypic effect was found with respect to: plant height, branch number, seed number per pod and weight of 100 seeds. However, the effect varied with the combination and year. It was observed that plant traits of the F_4 from the PI 238920 x Oyachi No. 2 were far more modified by the environment than those from the PI 238920 x PI 180517. Markedly low heritability coefficients were noted for earliness and yield parameters (Table 2).

Despite the equivocal genotypic and environmental determination of the set up of soybean traits in the F_3 and F_4, the heritability of yield structure elements showed higher values than the introduced forms had. Heritability coefficients of the latter for the number of seeds, seed weight, and weight of 100 seeds were estimated at 0.32, 0.33, and 0.46, respectively (Skorupska et al., 1984). The results suggest a good chance of developing genotypes with a more stable yield performance in hybrid generations and of their potential in the process of adjusting soybeans to new habitats.

Both the character and the range of correlation coefficients varied with years and cross combinations. Only a few correlations were found to occur with some regularity; e.g., in the F_3 of the two cross combinations, distinct relationship was noted for the number of branches and weight of 100 seeds. In the F_4, the height of plants was correlated with the number of seeds per plant (A: $r = 0.24$; B: $r = 0.17$) and with the number of seeds per pod (A: $r = 0.13$; B: $r = 0.16$) (Table 3). A similar relationship was recorded for earliness, branch number, and weight of 100 seeds. Earliness was negatively correlated with plant height and showed more distinctly in the experiment with F_4 plants

Table 2. Variation and heritability of traits in F_3 and F_4 in two soybean cross combinations

Traits / Cross combination	Mean		Variation coefficient		Heritability coefficient	
	A	B	A	B	A	B
			1st year of experiments			
Height, cm	55.4	73.1	9.2	11.5	0.63	0.90
Branch, number per plant	5.9	6.1	11.2	16.3	0.73	0.88
Seeds, number per plant	73.0	65.0	14.5	19.6	0.73	0.76
Seeds, number per pod	1.6	1.5	7.1	5.7	0.83	0.68
Seed weight per plant, g	9.4	10.9	11.7	18.7	0.46	0.66
Seed weight, g/100	12.8	16.5	9.7	7.7	0.91	0.62
			2nd year of experiments			
Earliness	11.0	12.1	24.1	21.4	0.76	0.16
Height, cm	75.7	98.5	9.0	11.7	0.93	0.89
Branch, number per plant	5.7	6.0	8.4	13.0	0.85	0.56
Seeds, number per plant	135.7	71.5	12.7	12.2	0.85	0.49
Seeds, number per pod	1.7	1.4	5.2	5.4	0.81	0.57
Seed weight per plant, g	20.7	12.9	13.0	10.7	0.85	0.34
Seed weight, g/100	15.3	18.2	19.4	8.5	0.94	0.89

Table 3. Phenotypic correlation coefficients in F_3 and F_4

Traits		1st year of experiments						
		1	2	3	4	5	6	
1. Height of plants	Cross Combination "A"	1.00	0.55	0.39	0.27	0.22	-0.34	Cross Combination "B"
2. Branch, number per plant		0.12	1.00	0.80	0.40	0.68	-0.21	
3. Seeds, number per plant		-0.34	-0.33	1.00	0.59	0.87	-0.27	
4. Seeds, number per pod		0.06	0.96	-0.40	1.00	0.61	0.02	
5. Seed weight per plant		-0.34	-0.33	0.82	-0.43	1.00	0.15	
6. Seed weight, g/100		0.39	-0.37	-0.31	-0.42	0.22	1.00	

		2nd year of experiments							
		1	2	3	4	5	6	7	
1. Earliness	Cross Combination "A"	1.00	-0.20	0.19	0.21	-0.30	0.09	-0.18	Cross Combination "B"
2. Height of plants		-0.45	1.00	0.41	0.17	0.16	-0.23	-0.52	
3. Branch, number per plant		0.10	0.04	1.00	0.71	-0.13	0.44	-0.48	
4. Seeds, number per plant		-0.34	0.24	0.02	1.00	-0.13	0.75	-0.55	
5. Seeds, number per pod		0.02	0.13	0.02	0.40	1.00	0.05	0.26	
6. Seed weight per plant		-0.64	0.30	0.06	0.63	-0.13	1.00	0.12	
7. Seed weight, g/100		-0.39	0.10	0.04	-0.39	-0.62	0.46	1.00	

from the PI 238920 x PI 180517 combination. Moreover, progenies of the latter exhibited that plants with a shorter growing period had a strong tendency to developing seeds with a higher weight of 100 seeds (A: r = -0.39; B: r = -0.18) (Table 3). The most distinct correlations with years and cross combinations were found between the elements of yield structure. Positive values for correlation coefficients were reported with respect to the weight of seeds and number of seeds per plant, in addition to weight of 100 seeds. Correlation values for the number of seeds and weight of 100 seeds were negative. The latter is worth mentioning in that selection for seed yield in our climate can possibly be more effective in progenies whose yield results from the number of seeds per plant rather than from the size of seeds.

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1) *Osmia rufa* L. (Apoidea, Megalichidae) - A potential pollinator in the subgenus *Glycine*

Studies on the biology of flowering of wild forms of the subgenus *Glycine* conducted in a controlled environment demonstrated a conspicuous drop-off of chasmogamic flowers within racemes (Skorupska et al., in press).

For pollination, a small-sized insect was employed, viz., a wild bee *Osmia rufa* L. (*O. bicornis* L.). The selected wild bee of the family Mechalichidae is described as the garden mason bee (Wójtowski, 1979). It belongs to the most common species of solitary bees that forage in the spring and occur all over Poland. The female is covered with dense hair colored rusty or ginger-red on the dorsal side. On the ventral side, the hair is bristly, yellowish-brown, and forms a so-called abdominal brush to gather nectar and pollen eventually used for pollination. The length of the body approximates 10-12 mm and that of the mouth organ 4.8 mm (Kugler, 1955).

Garden mason bees initiate foraging in the first ten days of April and complete it in the last part of June. Following the swarming period, female bees make their nests in natural conditions, i.e., look for shelter in cracks of buildings, dry-rot trees or empty stems of dried-off plants.

For pollination under a controlled environment, garden mason bees are easily available from rearing under trap nests (Wójtowski and Wilkaniec, 1969; Wójtowski, 1979). Housed trap nests are advised to be maintained at 4 C to restrain the bees from emergence and coordinate their foraging with the flowering period of plants.

There is considerable opportunity to adjust and control the flights of *Osmia rufa* L. Experiments with red flower demonstrated that pollination was postponed by three months and 60.3% of well-developed insects were obtained from the cocoons (Broda and Wilkaniec, 1980). Pollination of wild forms of the *Glycine* subgenus, experimentally delayed by two months and compared to pollination under natural conditions, showed the survival rate of garden mason bee to be decreased by 15%.

Osmia rufa L. is a polyphagous species and gathers nectar and pollen from blossoms of fruit trees and bushes, viz., apricots, peaches, cherries, plums, apples, pears, and black and red currants, raspberries, and blackberries. Also, garden mason bees tend to visit cultivated plants, such as winter rape, different varieties of vetch, red clover, and blossoms of hawthorn, blackthorn, wild rose, violets, etc. (Juga, 1962; Free and Williams, 1970; Tasei, 1973; Wójtowski and Feliszek, 1977). In addition, chasmogamic flowers of wild species of the subgenus *Glycine*, particularly of *G. tomentella, G. tabacina* and *G. falcata*, were observed to attract extensive visitations, as well as were preferred to bunches of flowers of red clover and bird's foot trefoil served to make sure the bees would not suffer from lack of food. As a result, the number of pods set by chasmogamic flowers was 3-4 times higher (Skorupska et al., in press).

Considering the easily manageable dates of foraging and the effect of pollination obtained so far, it is concluded that *Osmia rufa* L. is a prospective insect for controlled pollination in the subgenus *Glycine*.

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1) Observation on back mutation of white-flowered 'Wakashima' mutants.

In 1974, ten white-flowered soybean mutants and other different forms were obtained from approximately 6,894 M2 plants of purple-flowered soybean cultivar 'Wakashima' irradiated with 15 krad of gamma rays (Singburaudom, 1977). Seeds of each plant, especially of white-flowered mutants, were increased. Later, experimental lines were established and used in mutation experiments (Vipasrinimit, 1979; Noree, 1981).

In 1978, 316 seeds of 16 white-flowered plants derived from 6 lines of white-flowered Wakashima mutants were irradiated with a dose of 15 krad in the Gammator of the Department of Applied Radiation and Isotopes.

The irradiated seeds of each plant were immediately planted in single rows. About one week after planting, the number of germinated plants, with color of hypocotyl, were recorded. The number of seedlings were counted again in the second week. About 28 days after planting, the plants flowered. During three weeks of the flowering stage, the flower color of each plant was carefully observed and recorded. The results were 13 purple-flowered plants found among the total of 173 surviving plants (Table 1). Approximately 7% back mutation could be estimated.

In Table 1, the data show that the purple-flowered plants obtained were from three single plants originally derived from Wakashima mutant #4.

In continuing mutation experiments, it was found later that Wakashima lines previously derived from Wakashima mutant #5 (a sister line of Wakashima mutant #4) and other Wakashima mutant #109 produced various types of genetic variability, such as seed-coat colors (brown and black), hilum color, seed sizes (large, medium, and small) as well as seed-coat quality. Genetics of these Wakashima mutants is under investigation.

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Table 1. Appearance of purple-flowered plants in white-flowered 'Wakashima' mutants irradiated by gamma rays

Experimental lines		Number of seeds		Number of plants		
Origin	Line number	Irradiated	Germinated	At flowering stage	White flower	Purple flower
Wakashima mutant #2	78212/160-4	20	10	9	9	-
	78213/160-8	20	6	5	5	-
	78214/160-9	20	15	14	14	-
Wakashima mutant #3	78215/161-2	19	8	6	6	-
	78216/161-3	20	18	16	16	-
	78217/161-4	20	16	14	14	-
Wakashima mutant #4	78218/162-5	20	16	15	15	-
	78219/162-3	20	11	11	11	-
	78220/162-6	20	12	11	6	5
	78221/162-9	20	11	10	10	-
	78222/162-9	20	11	10	3	7
	78223/162-9	20	6	6	5	1
	78225/162-11	20	14	14	14	-
Wakashima mutant #6	78226/164-4	17	8	7	7	-
Wakashima mutant #8	78224/166-8	20	14	13	13	-
Wakashima mutant #9	78227/167-3	20	12	12	12	-
	Total	316	188	173	160	13

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1) Influence of genotype and growth stage on nitrogen fixation in soybeans.

One of the many characteristics that makes soybeans a desirable crop is their ability to fix nitrogen. In order to enhance this ability, it is necessary to look at the variation among various cultivars throughout their life cycle.

Genetic studies for Spanish clover (*Desmodium sandwicense* E. Mey) by Pinchbeck et al. (1980) showed a significant difference among genotypes in their ability to fix nitrogen. These differences suggest a genetic variation for nitrogen fixation in Spanish clover. Nitrogen fixation rates also vary with stages of the plant's growth. Hardy et al. (1968) reports that nitrogen fixation activity per plant in soybeans (*Glycine max* L. Merr.) is low prior to flowering, increases rapidly after flowering and decreases rapidly as the plant approaches the green bean stage.

Our objectives were to: 1) screen the 20 soybean varieties in Table 1 for nitrogen fixation potential, and 2) to determine the differences in nitrogen fixation among growth stages V4 (4 nodes on the main stem), R1 (at least 1 flower at each node), and R6 (pod containing full size green beans at one or more of the 4 uppermost nodes (Fehr et al., 1971).

Table 1. Maturity groupings of soybean cultivars tested for influence of genotype and growth stage on nitrogen fixation

Soybean cultivars				
MG IV	MG V	MG VI	MG VII	MG VIII
RA401	Bedford	Tracy	Braxton	Foster
RA480	Forrest	Davis	Hutton	Wright
Stevens	Wilstar 550	Centennial	Bragg	
	Essex	McNair 600		
	Bay	Greenseed 737		
		Lee		

Soybean cultivars were planted in sterile Dispo growth pouches and inoculated with USDA *Rhizobium japonicum* strains 3I1B 6 and 3I1B 122. Nutrients were supplied by Fahreaus nitrogen-free nutrients solution. The plants were

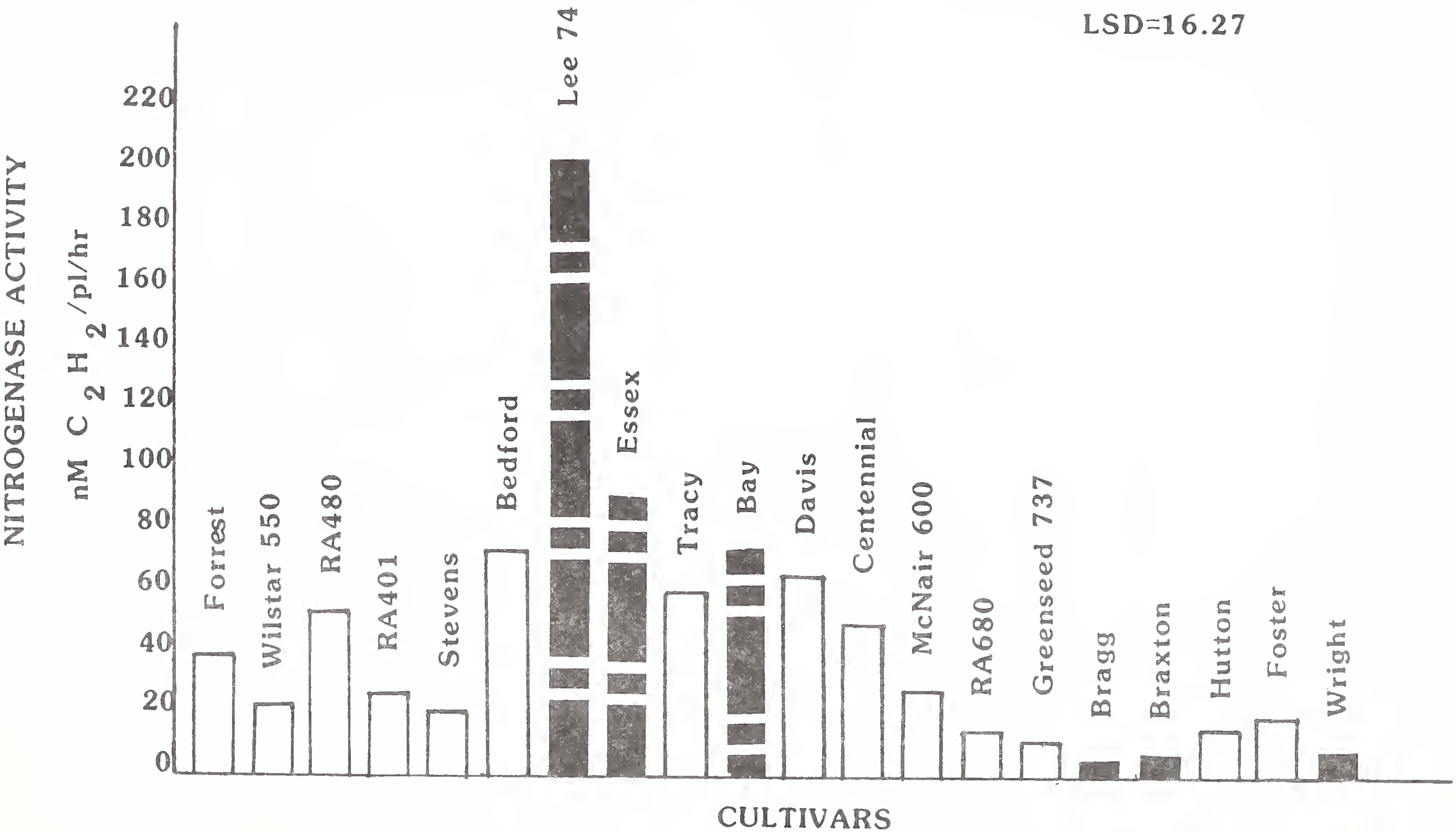

Fig. 1. Variation in nitrogenase activity among twenty commercial soybean cultivars.

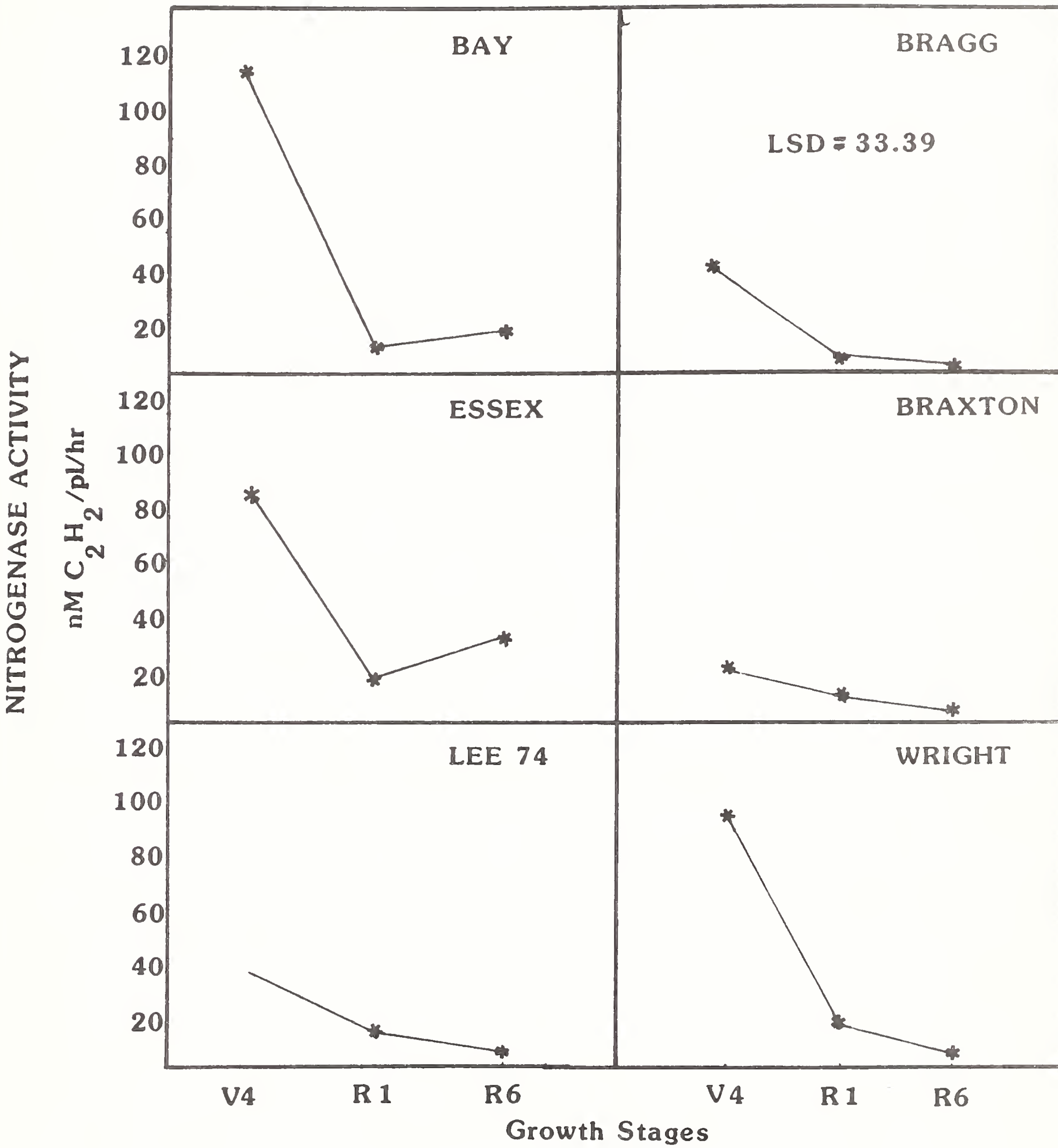

Fig. 2. Profiles of nitrogenase activity of six soybean cultivars.

grown in a growth chamber at 75 ± 5°F at 16 hr days for 35 days. They were arranged in a randomized complete block with 4 replications.

From the screening 'Bay', 'Essex', and 'Lee 74' were chosen as the highest nitrogen fixers and 'Braxton', 'Wright', and 'Bragg' were chosen as the lowest nitrogen fixers based on acetylene reduction. These cultivars were grown in the greenhouse in pro-mix A and inoculated with a commercial peat inoculant. Each cultivar was grown to the V4, R1, and R6 stage of growth. They were grown at 75 ± 5°F at a 16 hr light period. Arrangement was a split-plot design with four replications.

Results and discussion. Acetylene reduction revealed a significant variation in nitrogen fixation for the 20 cultivars (Fig. 1). There was a range in means per cultivar from 3.00 to 201.00 nMC_2H_2/pl/hr. There was a correlation among acetylene reduction and nodule number, nodule weight, and shoot fresh weight.

Seasonal variation among the cultivars Bay, Essex, Lee 74, Braxton, Wright, and Bragg was highly significant. The highest activity for all cultivars was at the V4 stage. As shown in Figure 2, nitrogenase activity decreased both at the R1 and R6 stages in all cultivars except Bay and Essex. Bay and Essex showed signs of recovery at the R6 stage.

Cultivars Bay, Wright, and Essex showed the highest activity at the V4 stage. These three cultivars also showed the highest activity at the R1 and R6 stages.

There seems to be significant variation in nitrogen fixation among cultivars. This variation continues throughout the life cycle, but the variation is most significant at the period before flowering (V4). After flowering, there is a continued decrease in nitrogenase activity.

Our ultimate goal in this area of research is to enhance nitrogen fixation in soybeans. Perhaps, through breeding, the decline in nitrogen fixation after flowering can be prevented, thus providing more nitrogen to the plant during seed production.

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1) The genomes of the genus *Glycine*

Based upon morphology, geographical distribution, isozyme studies and meiotic chromosome behavior in intra- and interspecific hybrids, we tentatively have assigned the following diploid (2*n*=40) genome designations to species in the genus *Glycine*.

Species	Genome
Subgenus *Glycine*	
1. *G. argyrea* Tind.	-
2. *G. canescens* F. J. Herm.	AA
3. *G. clandestina* Wendl. (long pod)	-
G. clandestina Wendl. (intermediate pod)	A_1A_1
G. clandestina Wendl. (short pod)	BB
4. *G. cyrtoloba* Tind.	CC
5. *G. falcata* Benth.	-
6. *G. latifolia* (Benth.) Newell & Hymowitz	B_1B_1
7. *G. latrobeana* (Meissn.) Benth.	-
8. *G. tabacina* (Labill.) Benth.	B_2B_2
9. *G. tomentella* Hayata	DD
Subgenus *Soja*	
10. *G. soja* Sieb. & Zucc.	GG
11. *G. max* (L.) Merr.	GG

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1) Screening progeny of mutagen-treated soybean seeds for nonfluorescent root mutants.

Delannay and Palmer (1982) reported four nonallelic mutants, three recessive and one dominant, that controlled root fluorescence in soybean. It was during this investigation that we became interested in looking for mutagen-induced nonfluorescent mutants.

In the fall of 1980, we wrote to various soybean researchers who were engaged in mutagenesis programs. The results of screening progeny of mutagen-treated seeds for nonfluorescent root mutants are given in Tables 1-5.

Seeds either were planted in a sandbench or germinated as for mitotic chromosome preparation (Palmer and Heer, 1973) and seedlings were examined with a UV light. All seedlings that were suspected to be nonfluorescent were transplanted to pots and grown in the greenhouse. The self-pollinated progeny were examined with a UV light. If all progeny of a plant had nonfluorescent roots, the original plant was called a "confirmed" nonfluorescent root mutant. In several cases, all progeny gave fluorescent roots, which indicated that an error had been made and the original plant had fluorescent roots.

Table 1 presents results from mutagen-treated seeds obtained from the University of Illinois. A total of 12 confirmed nonfluorescent mutants was found. Two suspected nonfluorescent mutant plants are growing and progeny will be examined for root fluorescence when the plants are mature. Two suspected nonfluorescent plants gave no progeny. One plant was yellow and died as a seedling; another plant was sterile and produced no progeny. Confirmed mutants were obtained from fission neutrons, gamma rays, and nitrosomethyl urea treatments. A total of 65,554 seedlings were examined.

Table 2 presents results from sodium azide-treated seeds obtained from Purdue University. No nonfluorescent mutants were obtained among the 3,217 seedlings examined.

Table 3 presents results from sodium azide and ethylmethane sulfonate-treated seeds obtained from Iowa State University. No nonfluorescent mutants were obtained among the 51,670 seedlings examined.

Table 1. Mutagen-treated seeds - Illinois

Treatment	Cultivar	Generation	No. of seedlings	Root fluorescence results
1.6kR (fission neutrons)	Harosoy rj_1 rj_1	M2	5222	5220 fluorescent 2 confirmed nonfluorescent
1.6kR (fission neutrons)	Williams	M2	5014	All fluorescent
2.2kR (fission neutrons)	Harosoy rj_1 rj_1	M2	4955	All fluorescent
2.2kR (fission neutrons)	Williams	M2	6794	All fluorescent
20kR (gamma rays)	Harosoy rj_1 rj_1	M2	3975	All fluorescent
20kR (gamma rays)	Williams	M2	5262	5260 fluorescent 2 confirmed nonfluorescent
25kR (gamma rays)	Harosoy rj_1 rj_1	M2	3084	All fluorescent
25kR (gamma rays)	Williams	M2	4296	All fluorescent
Ethylmethane sulfonate-1*	Williams	M2	6077	All fluorescent
Ethylmethane sulfonate-2**	Williams	M2	7321	All fluorescent
NMU-1^{+}	Williams	M2	6535	6528 fluorescent 4 confirmed nonfluorescent; 2 suspected nonfluorescent; 1 suspected nonfluorescent, was yellow and died.
NMU-2‡	Williams	M2	7019	7014 fluorescent 4 confirmed nonfluorescent; 1 suspected nonfluorescent, was sterile, no self- or cross-pollinated seeds were obtained.

*50 mM ethylmethane sulfonate for 9 hr, 9 hr postwash.
**50 mM ethylmethane sulfonate for 9 hr, 5 hr postwash.
$^{+}$2.5 mM nitrosomethyl urea for 5 hr, 9 hr postwash.
‡2.5 mM nitrosomethyl urea for 5 hr, 5 hr postwash.

Table 2. Mutagen-treated seeds - Indiana

Treatment	Cultivar	Generation	No. of seedlings	Root fluorescence results
Sodium azide*	Amsoy 71	M2	3217	All fluorescent

*10 mM for 2 hr.

Table 3. Mutagen-treated seeds - Iowa

Treatment	Cultivar	Generation	No. of seedlings	Root fluorescence results
Ethylmethane sulfonate*	Beeson	M3	4230	All fluorescent
Ethylmethane sulfonate	Corsoy	M3	7308	All fluorescent
Ethylmethane sulfonate	Hardin	M3	10,692	All fluorescent
Ethylmethane sulfonate	Pella	M3	3348	All fluorescent
Ethylmethane sulfonate	Weber	M3	2772	All fluorescent
Sodium azide**	Coles	M3	4417	All fluorescent
Sodium azide	Hardin	M3	8840	All fluorescent
Sodium azide	Pride B216	M3	2232	All fluorescent
Sodium azide	Weber	M3	7831	All fluorescent

*25 mM ethylmethane sulfonate for 9 hr.

**1 mM sodium azide for 2 hr.

Table 4. Mutagen-treated seeds - North Carolina

Treatment*	Cultivar	Generation	No. of seedlings	Root fluorescence results
Ethidium bromide	Jackson	M2	188	All fluorescent
Ethidium bromide	Lee	M2	168	All fluorescent
Ethidium bromide	Ransom	M2	198	All fluorescent
Ethidium bromide	Ransom	M3	450	All fluorescent
Ethidium bromide	Ransom	M5	276	All fluorescent

*Seeds were soaked for 3 hr in the dark in 0.25-0.50% ethidium bromide.

Table 5. Mutagen-treated seeds - Tennessee

Treatment	Cultivar or PI	Generation	No. of seedlings	Root fluorescence results
2.0kR (fission neutrons)	Essex	M5	1757	All fluorescent
2.0kR (fission neutrons)	Lee 74	M5	454	All fluorescent
2.0kR (fission neutrons)	Ogden	M5	638	All fluorescent
20kR (gamma rays)	Essex	M5	9356	All fluorescent
20kR (gamma rays)	Essex	M6	6291	All fluorescent
20kR (gamma rays)	Forrest	M6	175	All fluorescent
20kR (gamma rays)	Ogden	M5	180	All fluorescent
20kR (gamma rays)	Pickett 71	M6	536	All fluorescent
EMS*	Bedford	M3	557	All fluorescent
EMS	Bedford	M4	780	All fluorescent
EMS	Centennial	M3	572	All fluorescent
EMS	Centennial	M4	415	All fluorescent
EMS	Essex	M5	915	All fluorescent
EMS	Essex	M6	7525	All fluorescent
EMS	Forrest	M6	711	All fluorescent
EMS	Pickett 71	M6	368	All fluorescent
EMS	Ogden	M5	650	All fluorescent
EMS	PI 88788	M7	415	All fluorescent

*50 mM ethylmethane sulfonate for 8 hr.

Results from ethidium bromide-treated seeds from North Carolina State University are given in Table 4. No nonfluorescent mutants were obtained among the 1,280 seedlings examined.

Table 5 gives results from mutagen-treated seeds obtained from the University of Tennessee. No nonfluorescent mutants were obtained among the 32,295 seedlings examined.

A total of 154,016 seedlings were examined. Twelve confirmed and four suspected (includes the two that gave no seeds) nonfluorescent mutants were identified. Two confirmed mutants, designated IL 3-1 and IL 3-2, have been studied genetically and the results are given in the following article. The remaining 10 confirmed mutants will be studied genetically.

A summary of the number of seedlings examined according to mutagen is:

Treatment	
Ethylmethane sulfonate	54,656
Gamma rays	33,155
Sodium azide	26,537
Fission neutrons	24,834
Nitrosomethyl urea	13,554
Ethidium bromide	1,280
Total	154,016

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2) Genetic studies with two mutagen-induced nonfluorescent root mutants.

In the preceding article, we described several nonfluorescent root lines that had been obtained from induced mutagenesis. Seeds of the cultivar 'Williams' had been treated with 20kR gamma rays. The seeds given to us were a bulk harvest of many M2 plants. Only two nonfluorescent seedlings, designated IL 3-1 and IL 3-2, were found among the 5,262 seeds germinated. These two nonfluorescent lines were studied genetically and the results are given in this report.

The two nonfluorescent plants were crossed with each other and gave F_1 plants with fluorescent roots; the F_2 segregation fit a 9 fluorescent:7 nonfluorescent ratio (Table 1).

Table 1. Root fluorescence of F_1 plants and F_2 progenies from crosses between two unknown nonfluorescent soybean mutants, IL 3-1 and IL 3-2

	IL 3-2			
		F_2 segregation		
		Number of plants		
Unknown fluorescent line	F_1 †	Fluorescent	Nonfluorescent	χ^2* (9:7)
IL 3-1	F	143	106	0.14

†F means fluorescent roots.

*χ^2 of 3.84 is significant at the 0.05 probability level.

When the same two nonfluorescent plants were crossed to lines with fluorescent roots, the F_1 plants invariably had fluorescent roots, and the F_2 segregation fit a 3 fluorescent:1 nonfluorescent ratio (Table 2).

The two nonfluorescent plants both were crossed to the four standard nonfluorescent lines described by Delannay and Palmer (1982). With crosses involving IL 3-1, F_1 and F_2 data indicate that a locus different from that of the four standard nonfluorescent lines was responsible for nonfluorescence (Table 3). This new mutant nonfluorescent line (IL 3-1) was assigned Genetic Type Collection Number T280 and the gene symbol fr_5 by the Soybean Genetics Committee.

With crosses involving IL 3-2 and PI 290136, F_1 and F_2 data gave all nonfluorescent plants, indicating that IL 3-2 and PI 290136 possess the same

Table 2. Root fluorescence of F_1 plants and F_2 progenies from crosses between two unknown nonfluorescent soybean mutants, IL 3-1 and IL 3-2, and two fluorescent lines

	IL 3-1				IL 3-2			
		F_2 segregation				F_2 segregation		
		Number of plants				Number of plants		
Fluorescent lines	F_1 †	Fluorescent	Non-fluorescent	χ^2 (3:1)*	F_1 †	Fluorescent	Non-fluorescent	χ^2 (3:1)*
T272H	F	200	71	0.21	F	155	49	0.01
Hark	F	325	107	0.01				

†F means fluorescent roots.

*χ^2 of 3.84 is significant at the 0.05 probability level.

Table 3. Root fluorescence of F_1 plants and F_2 progenies from crosses between two unknown nonfluorescent soybean mutants (IL 3-1 and IL 3-2) and the four standard nonfluorescent lines

	IL 3-1				IL 3-2			
		F_2 segregation				F_2 segregation		
		Number of plants				Number of plants		
Non-fluorescent lines	F_1†	Fluorescent	Non-fluorescent	χ^2 (9:7)* or (3:13)	F_1†	Fluorescent	Non-fluorescent	χ^2 (9:7)* or (3:13)
Minsoy (fr_1 fr_1)	F	219	168	0.02	F	170	138	0.14
PI 290136 (fr_2 fr_2)	F	207	157	0.06	NF	0	230	
PI 404165 (fr_4 fr_4)	F	192	151	0.01	F	235	179	0.04
PI 424078 (Fr_3 Fr_3)	NF	68	266	0.57	NF	32	158	0.46

†F means fluorescent roots; NF means nonfluorescent roots.

*χ^2 of 3.84 is significant at the 0.05 probability level.

gene for lack of root fluorescence (Table 3). Both parents are white flowered and have tawny pubescence, but PI 290136 has black seed coat, whereas IL 3-2 has yellow seed coat. The hybridity of the original cross was confirmed by observation of seed coat color of F_3 seeds on different F_2 plants. Crosses of IL 3-2 with the other three nonfluorescent standard lines gave segregation for fluorescent and nonfluorescent roots (Table 3).

F_2 field-grown plants of the cross Hark x IL 3-1 were single-plant threshed. Twenty seeds from each of the 200 plants were germinated and tested for root fluorescence. Data indicated that 48 plants were true breeding nonfluorescent and, thus, were genotype fr_5 fr_5. A total of 53 plants were true breeding fluorescent (genotype Fr_5 Fr_5) and 99 plants segregated about 3:1 for fluorescence:nonfluorescence (χ^2 = 0.92), confirming the heterozygous genotype Fr_5 fr_5. The F_2 genotypic ratio was a close fit to the expected 1:2:1 (χ^2 = 0.27), which confirmed that nonfluorescence of IL 3-1 is conditioned by a single-gene recessive.

F_2 linkage tests were conducted between fr_5 and w_1 (Table 4). Percentage recombination was obtained from the ratio of products method (Immer and Henderson, 1943). Data indicated no linkage between fr_5 and w_1.

Table 4. F2 linkage test between IL 3-1 (w_1 w_1 fr_5 fr_5) and soybean cultivar Hark (W_1 W_1 Fr_5 Fr_5)

Genes	a	b	c	d	Sum	% R ± SE*	Linkage phase**
W_1 w_1 Fr_5 fr_5	269	92	92	31	484	50.2 ± 3.4	C

*% R ± SE = percent recombination ± standard error.

**C = coupling.

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3) Nucleolus distribution in quartets from diploid and triploid soybean.

There are few studies on inheritance and behavior of nucleoli in soybean. Yamaha and Sinoto (1925) reported the behavior of nucleoli in somatic mitosis of 30 species of higher plants including *Glycine max* [*Soja*]. Palmer and Heer (1976) observed one large nucleolus and six small nucleoli in a 40-chromosome plant from the st_4 st_4 synaptic mutant. Folsom and Peterson (1984), in ultrastructural studies of soybean embryo sacs, noted that a micronucleolus often was associated with the nucleolus of an egg cell. In our study, observations on nucleoli distribution at tetrad stage of meiosis were conducted on meiocytes of male-fertile and male-sterile diploids derived from male-sterile ms_1 ms_1 progeny and of male-fertile and male-sterile triploids.

In diploids, only one nucleolus generally was found in the meiocytes at early stages of meiosis. As shown in Table 1, nucleoli distribution in the quartet microspores in both male-fertile and male-sterile diploid plants fell into three major classes: 1-1-1-1, 1-1-1-2, and 1-1-2-2. Frequency among these three classes differed from each other between male-fertile and male-sterile diploid plants. Furthermore, about 16% of the quartets in male-sterile diploids had more than two nucleoli in one microspore or more than two microspores in one quartet with two nucleoli, compared with only 1.2% in male-fertile diploids. Frequently, the nucleoli varied in size.

In triploids, the meiocytes generally have one nucleolus. Infrequently, one large and one small nucleolus were observed. Nucleoli distribution in quartet microspores of both male-fertile and male-sterile triploid plants followed the same pattern as that of diploid male-sterile plants, rather than that of diploid male-fertile plants (Table 1). Although the above three main patterns of nucleoli distribution in quartet microspores were observed in both

Table 1. Frequency and distribution of nucleoli in tetrad stage of meiosis in triploid and diploid soybeans

Type of distribution	Triploid plants				Diploid plants			
	Male fertile		Male sterile		Male fertile		Male sterile	
	No. of quartets	%	No. of quartets	%	No. of quartets	%	No. of quartets	%
1-1-1-1	143	39.2	75	39.9	192	71.1	123	39.7
1-1-1-2	85	23.3	44	23.4	57	21.1	85	27.4
1-1-2-2	88	24.1	37	19.7	18	6.6	52	16.8
1-1-1-3	9	2.5	5	2.6	1	0.4	7	2.3
1-1-2-3	9	2.5	1	0.5	1	0.4	8	2.6
1-1-2-4	1	0.3	2	1.1	-	-	-	-
1-1-3-3	1	0.3	-	-	-	-	1	0.3
1-1-3-4	1	0.3	-	-	-	-	-	-
1-2-2-2	13	3.5	7	3.7	1	0.4	15	4.8
1-2-2-3	5	1.3	6	3.2	-	-	13	4.2
1-2-3-3	-	-	2	1.1	-	-	-	-
1-3-3-3	-	-	3	1.6	-	-	-	-
1-2-3-4	-	-	1	0.5	-	-	-	-
2-2-2-2	8	2.2	3	1.6	--	-	3	1.0
2-2-2-3	2	0.5	2	1.1	-	-	1	0.3
2-2-3-3	-	-	-	-	-	-	2	0.6
Total	365	100.0	188	100.0	270	100.0	310	100.0

male-fertile and male-sterile diploid and triploid plants, frequency of meiocytes with only one nucleolus in each member quartet (1-1-1-1) in male-fertile diploid plants seems much higher than those of male-sterile diploids, and male-fertile and male-sterile triploids (71.1% vs. 39.7%, 39.2%, and 39.9%, respectively), while the frequency of two members with one nucleolus and the other two members with two nucleoli in each quartet (1-1-2-2) in male-fertile diploids seems much lower than those of male-sterile diploids, male-fertile and male-sterile triploids (6.6% vs. 16.8%, 24.1%, and 19.7%, respectively)(Table 1). The occurrence of more than one nucleolus in members of quartets from diploid meiocytes indicates that there might be more than

one genome or perhaps more than one locus involved in formation of the nucleolus. Sybenga (1972) noted that, in principle, many loci are capable of organizing nucleoli, but that under normal conditions only the major nucleolar organizer performs this function and suppresses all other loci. Sybenga (1972) also pointed out that suppression of nucleolar organization may be observed when genomes of different species are combined. Cultivar soybean (*Glycine max*), which behaves cytogenetically and genetically as a diploid, has been suggested to be a tetraploid (Hadley and Hymowitz, 1973). Therefore, if soybeans were of polyploid origin, two or more genomes might be involved in formation of the nucleolus. The failure of suppression of nucleolus formation in the other genome by the major genome might result in two or more nucleoli in one microspore.

Genomic unbalanced gametes are expected from the triploid meiocytes and the existence of four nuclei in coenocytic microspores might also have some effect on the normal formation of the nucleolus. These might lead to the variation in frequency of nucleoli distribution among classes between the male-fertile diploids and the other three sources (male-sterile diploids, male-fertile and male-sterile triploids). Frequently, small scattered nucleoli also were observed in some coenocytic microspores of both diploid and triploid male-sterile plants. Nevertheless, nucleoli in each member of most quartets tended to fuse before pollen development.

McClintock (1934) first reported that the development of the nucleolus is associated with the nucleolar organizing element in the satellite chromosome of maize and that the number of nucleoli in the resting nucleus generally is in proportion to the number of normal satellite chromosomes. Givens and Phillips (1976) used partial triploids and tetraploids of the nucleolar organizer region (NOR) to study the nucleolar distribution throughout meiosis in maize. They found that the site giving rise to the secondary constriction can organize a nucleolus and that duplication of this segment can result in formation of two nucleoli. In diploid soybean (2n=40), only one pair of satellite chromosomes was identified in our laboratory. Pillai (1976) noted a 2n=40 large seed variety with four satellite chromosomes in soybean (*Glycine max*). However, meiosis study was not done in their study. Zheng et al. (1984) also reported four satellite chromosomes in a diploid strain of wild soybean (*Glycine soja*). Previously, Biswas and Bhattacharyya (1972) reported that there are four pairs of chromosomes bearing secondary constrictions in

diploid soybean. Whether all these four pairs of chromosomes with secondary constrictions are associated with nucleolus formation is not known. Our study indicated that there might be more than one pair of chromosomes involved in formation of the nucleolus. Since the plants used in this study all derived from male-sterile ms_1 ms_1 progeny, further investigation on normal diploid genotypes is necessary to preclude material specificity or the effect of male-sterile ms_1 locus on nucleolus formation.

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1) Screening soybean genotypes for iron-deficiency chlorosis in the growth chamber using potted soil.

Iron-deficiency chlorosis in soybean is a persistent problem on calcareous soils of the upper Midwest. Substantial genetic variability for tolerance to iron-deficiency chlorosis has been found among popular varieties and in the germplasm collection. Due to significant breeding efforts, high-yielding varieties that are tolerant to iron-deficiency chlorosis are now available. These efforts, however, have been hampered by difficulties in screening for iron-deficiency chlorosis in the field. Previous attempts to use potted soil to screen for iron-deficiency chlorosis have not been successful (Byron and Lambert, 1983). Recently, Coulombe et al. (1984) have developed a successful screening method using nutrient solution. We would like to report some initial attempts at achieving an inexpensive and labor-efficient method for screening soybean genotypes for iron-deficiency chlorosis using potted soil in the growth chamber.

Materials and methods. Soil was collected from four different sites, all within the Nicollet-Webster broad soil classification, near the following cities in southern Minnesota: Bechyn, Hanska, Lamberton, and Wilmar. An area approximately 10 m in diameter was selected where severe iron-deficiency chlorosis had been observed in previous years at each of the sites. Approximately 300 kg of soil (field-moist) was collected from the upper 0.5 m of soil from each of the areas and placed in plastic bags. In the laboratory, each soil (field-moist) was sieved through a 1 cm screen and mixed thoroughly. Weights of a small sample (approx. 500 g) of each soil were determined before and after drying at 105 C for 24 hrs and θ_w (gravimetric moisture content) for each soil was obtained using the equation θ_w = (dry weight - wet weight)/dry weight. Soil was packed evenly to a volume of 2.0 liter and a bulk density of 1.1 in 2.4-liter plastic, undrained (no bottom hole) containers. Eight seeds of a single genotype were planted in each container to a depth of approximately 2 cm below the soil surface. Three hundred g of dry, washed sand was placed on the soil surface as a mulch. Pots were placed in a growth chamber with a 16 hr day, 23 C day, 20 C night, and were watered lightly each day until the seedlings had reached the unifoliate (V1) stage (approximately 10 days

after planting). Plants were then thinned to three plants per pot and the soil brought to a predetermined water content by adding water until each pot had reached a specific weight. Soil water content was maintained constant by weighing each pot every 24 hr and adding sufficient water to compensate for evapotranspiration. Visual chlorosis scores were taken on individual plants when the second trifoliate had fully expanded.

The following genotypes were selected to represent a range from the most tolerant to the most susceptible genotypes available: A2, A7, 'Anoka', 'Chlorosis Tolerant Anoka', 'Corsoy 79', 'Dawson', 'Hodgson 78', 'Pride B216', 'Simpson', 'Swift', T-203, and 'Weber'. Screening experiments were conducted separately for each of the four soils. There were three pots (replications) per genotype with three plants per pot arranged in a completely randomized design with subsampling. Soil water content (θ_w) was maintained at 0.42 for the Hanska, Lamberton, and Wilmar soils, and at 0.55 for the Bechyn soil.

A field nursery was planted at the Hanska site during the summer of 1984. The above-mentioned genotypes were planted together with breeding material from the University of Minnesota soybean breeding program. Plots corresponded to single 1.3 m long rows with 76 cm-row spacing arranged in a randomized block design with three replications. Visual readings were taken at approximately the same growth stage as those in the growth chamber (full expansion of the second trifoliate).

Results and discussion. Results of initial experiments conducted in the growth chamber and the field are summarized in Table 1. Experiments just concluded indicate that the soil moisture content at which the most severe chlorosis occurred differed for each soil. The moisture contents used in the screening experiments with the Hanska, Lamberton, and Wilmar soils were below the appropriate levels for inducing the most severe chlorosis on those soils. For instance, raising the soil moisture content to 0.50 in the Hanska soil resulted in a chlorosis score of 5.0 for Corsoy 79, a value much closer to that observed in the field. We also found that the LSD values could be reduced by remixing the soil immediately before packing the pots, presumably due to moisture differences within the soil that occur during storage of the mixed soil. Current efforts are directed toward determining the optimum soil moisture content to induce the most severe chlorosis in each soil, reducing the variability observed among genotypes, and at improving the labor and cost efficiency of the procedure.

Table 1. Summary of results of initial experiments conducted in the growth chamber and the field

	Soil Source				Field Nursery
Genotype	Bechyn	Hanska	Lamberton	Wilmar	Hanska
A2	4.1	1.2	1.0	2.2	3.3
A7	2.2	1.0	1.0	1.1	1.3
Anoka	5.0	4.2	1.7	4.4	5.0
Anoka (tolerant)	3.0	1.8	1.3	1.9	3.3
Corsoy 79	4.8	2.8	2.2	3.3	4.7
Dawson	3.3	1.2	1.0	1.2	2.3
Hodgson 78	4.7	1.9	1.0	2.4	2.7
Pride B-216	5.0	4.9	2.4	3.9	4.8
Simpson	4.7	1.3	1.4	1.6	4.0
Swift	2.8	1.1	1.1	1.3	3.0
T-203	5.0	4.8	5.0	4.9	NI+
Weber	4.2	1.0	1.8	1.0	3.3
Mean	4.1	2.3	1.8	2.4	3.4
LSD 0.05	1.0	1.9	2.3	2.3	1.2

+NI = Not included in test.

Conclusions. The general procedure appears to work well as an inexpensive and labor-efficient method of adequately evaluating the relative efficiency of different genotypes for iron-deficiency chlorosis. The method also appears to work well using several different soils, provided that genotype comparisons are made using the same soil. Since appropriate moisture contents differ for each soil, we recommend that, once a soil is chosen, a range of soil moisture contents be tested using a moderately susceptible genotype (such as Corsoy 79) to determine which moisture content induces the most severe chlorosis on that soil. An appropriate range of moisture contents to test would be from 0.35 to just below saturation (between 0.50 and 0.60 for most soils) in increments of 0.05.

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1) SG1 - A recently constructed random-mated soybean population possessing the ms_2 gene for genetic male sterility

Soybean breeders and geneticists may be interested in a soybean population that we constructed and recently released on 1 February 1985. This population, called 'SG1', originated from 156 parental matings and has been random-mated for three generations. SG1 segregates for male-fertile (MF) and male-sterile (MS) plants due to the presence of the ms_2 gene (genetic male sterility) in the population (Bernard and Cremeens, 1975; Brim and Stuber, 1973). The parental matings, the F_1 selfing generation, and the first random-mating of F_2 plants were accomplished at the Nebraska Agriculture Experiment Station. SG1 was then randomly divided into five sub-populations for the second and third random-matings, conducted cooperatively but independently by the Agriculture Experiment Stations of Nebraska, Maryland (Dr. W. J. Kenworthy), Minnesota (Dr. J. H. Orf), and Missouri (Dr. D. G. Helsel), and the Ohio Agricultural Research and Development Center (Dr. S. K. St. Martin).

The initial synthesis of SG1 was accomplished by making all possible two-way crosses between 39 female parental lines and four male parental lines. The pollen donors were Ms_2ms_2 plants that were selected from near-isogenic, male-sterile, maintainer lines of the adapted cultivars 'Beeson' (maturity Group II), 'Wells' (II), 'Williams' (III), and the genetic type 'T259H' (III). T259H was the original source of the ms_2 gene and was a progeny selection from the mating SL11 x L66L-177, where the latter was (essentially) the cross: Wayne X [Hawkeye X Lee]. The ms_2 gene had been previously introduced into the other three cultivars by backcrossing methods (Bernard and Cremeens, 1975).

Of the 39 female parents, 31 consisted of 29 ancestral plant introductions and two obsolete cultivars ('Capital' and 'Lincoln'). These 31 strains were chosen on the basis of their frequent occurrences in the pedigrees of public soybean cultivars developed in hybridization programs during the period 1939 to 1980 (Specht and Williams, 1984). Six of the 39 parents were near-isogenic lines of two cultivars ('Harosoy' and 'Clark'). These isolines possessed genes conditioning morphological traits of purported agronomic worth (Hartung et al., 1980), namely Dt_2 (semideterminate stem habit), S (short main stem internodes), Pd_1 (dense plant pubescence), and pa_1 pa_2 (appressed plant pubescence). The remaining two female parents were two other plant

introductions ('Manchuria 13177' and PI 360.844) that were unrelated to the other parents. The latter PI strain (also known as 'Raiden') is purported to have a faint floral fragrance attractive to honey bees (Erickson, 1975). The 39 female parents (with respective maturity groups) are listed below:

Manitoba Brown	(00)	Korean	(II)	Aoda	(IV)
PI 194654	(00)	Mukden	(II)	Clark-Dt_2 S	(IV)
Capital	(0)	Richland	(II)	Clark-Pd_1	(IV)
Mandarin (Ottawa)	(0)	Seneca	(II)	Clark-pa_1 pa_2	(IV)
PI 180501	(0)	PI 65338	(II)	Midwest	(IV)
Habaro	(I)	A.K. (Harrow)	(III)	Patoka	(IV)
Mandarin	(I)	Dunfield	(III)	Peking	(IV)
Sac	(I)	Illini	(III)	Sato-3	(IV)
Bansei (Ames)	(II)	Jogun (Ames)	(III)	Arksoy	(VI)
Harosoy-Dt_2 S	(II)	Lincoln	(III)	Haberlandt	(VI)
Harosoy-Pd_1	(II)	Manchu	(III)	Hahto	(VI)
Harosoy-pa_1 pa_2	(II)	Manchuria 13177	(III)	Ogden	(VI)
Kanro	(II)	PI 360844	(III)	Roanoke	(VII)

About 5 to 10 F_1 seed from the 156 parental matings were generated by hand-pollinations during the period 1978 to 1981. Female parents were emasculated only for those matings where genetic markers were not available to verify F_1 authenticity. The F_1 plants (genotypically $1Ms_2$ Ms_2:$1Ms_2$ ms_2) were selfed and individually threshed to obtain F_2 seed.

For the first random-mating of SG1 in 1982, two randomly selected F_2 seeds from each of the 156 matings were composited for planting into a 2-row field plot (12-m length, 76-cm row spacing) in an isolated intermating nursery. There was sufficient F_2 seed to plant a total of 240 plots. This seed-compositing procedure ensured that F_2-plant representatives of each of the 156 parental matings would be in close proximity (within each field plot). This was done to minimize possible spatial limitations imposed on random-mating as a consequence of short-distance, insect-mediated, pollen transfer from MF plants to MS plants. One-half of the 240 field plots were planted in a north-south row direction, with the remaining 120 field plots planted about three weeks later over the top of the first planting in an east-west row direction. This dual-date planting procedure was used to provide a possibly greater overlap in the flowering periods of plants differing in maturity, since large differences in flowering dates can impose temporal limitations on random-mating early- and late-maturing plants. A honeybee hive was placed in the nursery to promote pollen transfer from MF to MS plants (Erickson, 1975).

The F_2 plants in the 1982 intermating nursery segregated 7MF:1MS ($5MS_2\ MS_2$:$2MS_2\ ms_2$:$1ms_2\ ms_2$). The reduced seed set of MS plants distinguished them from MF plants at maturity, but as a precaution, a large number of MS plants were identified and tagged at flowering for later identification at harvest (Brim and Kenworthy, 1977). MS plants bearing outcrossed seed were gathered at maturity and threshed in bulk. The bulked seed was then randomly subdivided into five equal portions for use by the five cooperating researchers in conducting the subsequent random-matings of SG1.

In the 1983 intermating nurseries, the plants segregated 6MF:1MS ($6Ms_2\ ms_2$:$1ms_2\ ms_2$). Each cooperator gathered and threshed in bulk the MS plants in their nurseries, using this bulked seed for advance to the third random-mating generation in 1984. Each cooperator also harvested seed (in bulk and/or by single-seed-descent) from the MF plants growing in the 1983 intermating nurseries, placing this seed in cold storage for subsequent distribution purposes.

In the 1984 intermating nurseries, the plants segregated 1MF:1MS ($1MS_2\ ms_2$:$1ms_2\ ms_2$). The harvest of MS and MF plants in 1984 was identical to that performed in 1983. No seed was exchanged among cooperators during any of the SG1 intermatings.

The seed obtained from the 1983 and 1984 harvests of MF plants in the SG1 sub-populations will be made available to interested soybean researchers after February 1, 1985. In both years, this seed was derived from heterozygous $Ms_2\ ms_2$ plants. Therefore, it should result in plants that segregate 3MF:1MS ($1Ms_2\ Ms_2$:$2Ms_2\ ms_2$:$1ms_2\ ms_2$). The cooperators avoided imposing any artificial selection during the intermatings; however, natural (and inadvertent artificial) selection at each cooperator location may have had different effects on each subpopulation, particularly with respect to the maturity range within these subpopulations.

The unique parental composition of the SG1 population (Specht and Williams, 1984), coupled with the facilitation of intermating by means of genetic male-sterility (Brim and Stuber, 1973), may make this population of interest to soybean breeders for use in empirical investigations of soybean response to recurrent selection (St. Martin, 1981). Furthermore, by interplanting recently released cultivars or elite breeding strains with SG1 in a common nursery, many natural matings of SG1 MS plants with these cultivars can be accomplished, thereby generating progeny that can be evaluated in

conventional breeding programs (Burton and Brim, 1977). Researchers interested in obtaining seed of any of the SG1 subpopulations (identified as NBSG1, MDSG1, MNSG1, MOSG1, OHSG1) should address written requests for seed to the authors or respective cooperators. Requests for reasonably large amounts of seed can be accommodated. We plan to continue random-mating SG1 without selection for several more generations; thus, seed from 1985 and later harvests may be used to honor future seed requests.

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1) Heterosis performance and combining ability in soybeans.

Before hybrid soybeans can become a reality, two requirements should be satisfied: (i) an economical large-scale method of producing hybrid seed must be found, and (ii) heterosis for yield must exist. With the findings of genetic male sterility in soybeans (Brim and Young, 1971) interest has developed in the potential productivity of hybrid soybeans. A method for producing experimental quantities of hybrid soybean seed using genetic male sterility and green seed embryo was suggested by Burton and Carter (1983). Studies have shown the average high-parent heterosis for yield of hybrid soybeans to range from 8% (Paschal and Wilcox, 1975) to 25% (Chaudhary and Singh, 1974). The objective of this study was to determine the magnitude of heterosis and combining ability for agronomic characters in soybeans adapted to Oklahoma.

Materials and methods: The study was conducted at the Agronomy Research Station, Perkins, Oklahoma, in the summers of 1982 and 1983. Six F_1 hybrids (all combinations except reciprocals) of the cultivars 'Douglas', 'Essex', 'Forrest', and 'York' were space-planted along with their parents in a randomized complete block design with four replications in 1982 and eight replications in 1983. The spacing between plants and between rows was the same (76 x 76 cm^2). Data collected were seed yield/plant (g), number of pods/plant, number of seeds/pod, seed size (weight in g of 100 random seeds), plant weight (g), harvest index, and height (cm). The statistical analyses were performed on entry-blocks means. In the analysis of variance, years, and genotypes were assumed fixed. The 1982 and 1983 tests were combined in the analyses.

Diallel analysis was obtained using Gardner and Eberhart (1966) analysis III. In this analysis, the among F_1 hybrids were partitioned into general (GCA) and specific combining ability (SCA), using Griffing's (1956) method 4 (parents excluded), model 1 (fixed model).

Results and discussion: Average midparent heterosis for yield, number of pods/plant, number of seeds/pod, seed size, plant weight, harvest index, and height were 24.6, 18.0, 0.4, 2.7, 19.5, 4.9, and 13.5%, respectively. Average high-parent heterosis for yield, number of pods/plant, number of seeds/pod, seed size, plant weight, harvest index, and height were 20.1, 6.9, -3.4, -7.5,

14.0, 1.7, and 7.6%, respectively. Five out of six hybrids were significantly ($P \leq 0.05$) higher in yield than the high-parent. The hybrid of Douglas/Essex expressed the highest heterosis response for yield with 37.1% for midparent heterosis and 32.5% for high-parent heterosis, and these were highly significant ($P \leq 0.01$). The hybrids of Douglas/Forrest expressed the lowest heterosis response for yield with 8.9% for midparent heterosis and 2.5% high-parent heterosis.

The years x parents component from the analysis of variance was significant for yield, number of pods/plant, number of seeds/pod, seed size, harvest index. The years x F_1s component, however, was significant only for harvest index. These results indicate that the hybrids were more stable over both years than their parents for yield, number of pods/plant, number of seeds/pod, and seed size.

Mean squares for GCA and SCA were significant for number of pods/plant and harvest index, suggesting the presence of additive and nonadditive genetic variance in this population for these characters. Mean squares for GCA were significant for number of seeds/pod, seed size, and height, suggesting the presence of additive genetic variance in this population for these characters. Mean squares for SCA were significant for yield and plant weight, suggesting the presence of nonadditive genetic variance in this population for these characters.

The years x GCA interaction was significant only for plant weight and harvest index. The years x SCA interaction was not significant for any characters evaluated. These results indicate that SCA was more stable over both years than GCA for plant weight and harvest index. The other characters appeared to be equally stable for both types of combining ability.

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2) Combining ability for seed protein and oil content in soybeans.

The diallel analysis technique allows the breeder to estimate the relative importance of general and specific combining ability for important traits in terms of the nature of gene action. Information on these systems is of value in the development of soybean hybrids as well as in the development of pure-line varieties. In this study, a primary objective was to determine the relative magnitudes of general and specific combining ability estimates for protein and oil content in four soybean cultivars grown in Oklahoma.

Materials and methods: The material for this study consisted of four parents ('Douglas', 'York', 'Essex', and 'Forrest'), six F_1s and six F_2 hybrids. The test materials were space-planted in a randomized complete block design in eight replications at the Agronomy Research Station, Perkins, Oklahoma, in the 1983 growing season. Percent seed protein and oil of each plant was estimated by the Technicon Infraanalyzer 400 (near-infrared reflectance). All diallel tests (F_1s and F_2s) were subjected to combining ability analysis using Model 1, Method 4 of Griffing (1956).

Results and discussion: The analysis of variance showed that significant differences are present for protein and oil content among F_1 hybrids and for protein content among F_2 hybrids indicating the presence of sufficient amounts of genetic variability for these traits in these soybean populations.

Highly significant general combining ability effects were observed for protein content in both generations and highly significant specific combining ability effects were observed for oil content in the F_1 generation. These findings are in agreement with those reported by Leffel and Weiss (1958) for oil but not for protein content.

Since general combining ability mean squares were significant only for protein content, general combining ability effects of parents were evaluated

with regard to this trait. Douglas had the greatest positive general effects in both generations, indicating that this parent tended to transmit high protein content to all progenies. Forrest had the greatest negative general combining ability effects in the F_1 generation indicating that this parent tended to transmit low protein content to all progenies. In the F_2, both Forrest and York had significant negative general combining effects.

Since specific combining ability mean squares were significant only for oil content in the F_1 generation, estimates of specific combining ability effects associated with individual crosses were evaluated for this trait. The hybrids Douglas x Forrest and Essex x York had the greatest positive specific effects (highest in oil content). These particular crosses would be potentially valuable in a breeding program where high oil is of prime consideration. The hybrids Douglas x York and Forrest x Essex had the greatest significant negative effect (lowest in oil content). The negative specific effects of the hybrids Douglas x Essex and Forrest x York were not significant.

In conclusion, the results obtained from the combining ability study indicated that general combining ability effects were important for protein content in both generations, while specific combining ability effects were important for oil content only in the F_1 generation. The line Douglas had the most promising general effect for protein content and the hybrids Douglas x Forrest and Essex x York had the most promising specific effects for oil content. Kempthorne and Curnow (1961) pointed out that general combining ability variance is due primarily to additive genetic variance, while specific combining ability variance estimates primarily nonadditive genetic variance. In this study, Griffing's (1956) Model I, Method 4 were utilized. In this model, the genotypes in the diallel are considered a fixed population and inferences are therefore valid only for the experimental material in the study.

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3) <u>The effect of the narrow-leaf gene in a segregating population.</u>

A single recessive gene designated *ln* by Bernard and Weiss (1972) controls the inheritance of the lanceolate trifoliolate in soybean. They state that the narrow-leaf condition is associated with a high number of four-seeded pods, which they attributed to a pleiotropic effect of the *ln* gene.

No yield differences have been found between narrow and normal leaf types using isolines. Where the narrow-leaf isolines have a higher number of seeds per pod, they have lower 100-seed weight or lower number of pods per plant compared with the normal-leaf isolines (Hartwig and Edwards, 1970; Mandl and Buss, 1981). No previous study has tested the *ln* gene in the heterozygote state. The objectives of this study were to examine the differences for yield and yield components in the F_2 *Ln/Ln*, *Ln/ln* and *ln/ln* genotypes in a cross between a narrow and a normal leaf cultivar.

<u>Materials and methods:</u> The parents selected for this study were the normal-leaf cultivar 'Douglas' and narrow-leaf cultivar 'Miles'. Miles has the T109 germplasm, a narrow-leaf line in the soybean genetic collection. The plants were space-planted (76 x 76 cm) in order to minimize interplant competition. Experimental design was a randomized complete block design consisting of ten replications. Experimental units were each individual plant. The study was conducted at the Perkins Agronomy Research Station (Teller Loam soil), Perkins, Oklahoma, in 1983.

Leaf length and leaf width were taken on the center leaflet of the most recent full expanded trifoliolate. The ratio of leaf width to leaf length was used to classify each plant as narrow (*ln/ln*), intermediate (*Ln/ln*) or normal (*Ln/Ln*) leaf type. Additional measurements were taken at harvest as follows: Plant height (cm), plant biomass (g), number of pods/plant, seed size (the weight of 100 random seeds in grams), number of seeds/pod, yield (g), and harvest index.

<u>Results and discussion:</u> The ranges of the ratio of leaf width to leaf length were 0.32 to 0.45, 0.52 to 0.65, and 0.65 to 0.87 for Miles, F_1, and Douglas, respectively. There was no overlap among the three genotypes. There were 70, 126, and 58 plants classified as narrow, intermediate, and normal, respectively, in the F_2 generation, using the above ratios. These numbers fit the expected 1:2:1 ratio ($P = 0.5$ to 0.7).

No differences in height and harvest index were found among the three genotypes, indicating that these characters were not associated with the *ln*

gene. However, significant differences were observed among the means of the narrow, intermediate, and normal genotypes for the characters: plant biomass, number of pods/plant, seed size, number of seeds/pod, and yield. Differences for number of seeds per pod (2.30, 2.44) and seed weight (14.1 g, 13.0 g) were observed between the normal and the narrow F_2 genotypes, respectively. No differences were observed for yield (85.6 g, 80.5 g), number of pods per plant (262, 250), or plant biomass (196.8 g, 182.3 g) between the normal and narrow F_2s, respectively. These results support conclusions of Mandl and Buss (1981) and Hartwig and Edwards (1970), who found that the *ln* gene was associated with a higher number of seeds/pod, and smaller seed size.

The intermediate (heterozygote) had significantly higher means for plant biomass (220.7 g), number of pods/plant (292) and yield (96.2 g) than either the narrow or the normal. Seed size (19.9 g) and number of seeds/pod (2.34) were not different from the normal leaf. These results suggest that there is an association between the *ln* gene and plant biomass, number of pods/plant and yield in the heterozygous condition. Thus, while there is a compensating effect in the yield components in the normal (*Ln/Ln*) and narrow (*ln/ln*) genotypes producing no yield differences, the intermediate *Ln/ln* genotype appears to be favored in the space-planting environment producing higher number of pods/plant, consequently higher yield.

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1) Subunit composition of glycinin from various samples of cultivated and wild soybean.

The major storage protein of 11S class of soybean seeds, glycinin, has a complex subunit structure. Each of the six subunits is composed of two protein molecules (acidic and basic), linked via disulphide bonds (Badley et al., 1975). Depending on subunit, the acidic moiety molecular weight varies from 37,000 to 42,000, with one exception (m.w. 10,000). By this parameter, the basic moieties of the subunits are more homogeneous (m.w. close to 20,000) (Moreira et al., 1979).

Plant storage protein genes are studied intensively. These genes seem to be an appropriate model for studying the regulation mechanisms of protein biosynthesis, gene structure, and evolution. It has been shown that multiple genes coding individual glycinin subunits may be grouped in several families (Goldberg et al., 1981). The variability of subunit structure within the corresponding protein families and the evolutionary interrelatedness of the subunits has not been studied in detail. On the basis of such data, mechanisms of soybean storage protein evolution may be elucidated, as well as the species specificity of its structure. Such information also is necessary for improving soybean agronomic traits, i.e., the methionine content, by selection.

In this paper, we compare the subunit composition of purified glycinin isolated from the seeds of various soybean cultivars and wild forms of these plants.

Materials and methods

Protein extraction and isolation of glycinin. Seed samples were obtained from the All-Union Institute of Plant Breeding (USSR, Leningrad). Protein extraction was carried out according to Hill and Breidenbach (1974). The protein extract (3-5 mg of protein in 200 μl of sodium phosphate buffer, containing 0.4 M NaCl and 10 mM of 2-mercaptoethanol, pH 7.5, buffer A) was layered on a sucrose density gradient (10-35% w/v, buffer A) was centrifuged for 18 hr at 36,000 r.p.m., rotor SW-41, "Beckman", at 20°C. Protein content in each of 30-40 fractions was estimated as described by Sedmac and Grossberg (1977), and

the relative content of 11S component was estimated by the area (weight) of the peak, corresponding to glycinin in the sedimentagram. In order to isolate glycinin, the total extract was fractionated with ammonium sulphate and isoelectric precipitation in 60 mM Tris-HCl (Wolf et al., 1962). After this procedure, glycinin (20-40 mg), containing admixtures of β-conglycinin and other proteins, was purified by passing through a column (1.5 x 10 cm) with concanavalin-A-sepharose ("Pharmacia") at a rate of 10 ml/hr in order to eliminate glycosylated admixtures. The protein of the first peak was precipitated with ammonium sulphate, dissolved and purified by passing through a column with DEAE-Sephadex A-50 (2.2 x 20 cm), as recommended by Mori et al. (1979). In the final stage, the material of 11S peak was separated by sucrose density gradient centrifugation under the conditions described above.

The amino acid analysis. Automatic amino acid analyzer Durrum D-500 was used. Glycinin samples were previously oxidated by performic acid for 4 hr, liophylized and then hydrolyzed by 5.7 M HCl for 24 hr at 110°C.

Electrophoresis and isoelectric focusing. Electrophoresis in 10% polyacrylamide gel with sodium dodecyl sulphate (SDS) was carried out according to the method of Laemmly (1970). Isoelectric focusing was carried out in "Multifor" ("LKB") in pH gradient 3.5 - 10 with 6 M urea for 4 hr at 1,000 V. Gel was stained as described by Jacle (1979).

Chromatography of glycinin subunits. Purified glycinin was reduced and subjected to S-alkylation with 4-vinylpyridin as described by Hermodsen et al. (1977). The reduced protein preparation was dialyzed against 50 mM sodium phosphate buffer with 6 M urea and 20 mM of 2-mercaptoethanol, pH 6.6. Chromatography (0.5-1.0 mg of protein) was carried out on "Mono Q2" column of fast protein liquid chromatography (FPLC) system ("Pharmacia"); the column was previously equilibrated with the same buffer. Elution was carried out with salt concentration gradient.

Results and discussion

The content of glycinin in the soybean varieties. Three major peaks with sedimentation coefficients 2S, 7S, 11S, respectively, and one minor peak (15S), corresponding to aggregated material, were observed. The amount of the aggregated material does not exceed 5%. Data on glycinin content based on the estimated proportion of the material of the 11S peak are presented in Table 1. The results demonstrate that glycinin content in most cultivated and semiwild varieties is 36-41%. The content of 11S protein in wild perennial species is lower.

Table 1. The content of the 11S component in different samples of soybean seeds (in % to extractable protein)

Sample No.	Cultivar	Species, subspecies	Content of 11S component, percent
1	Rannaja-10	*G. max*, ssp. *chinensis* Enk.	40.0
2	Ada	ssp. *manshurica* Enc.	40.0
3	Merit	ssp. *manshurica* Enc.	39.0
4	Richland	ssp. *manshurica* Enc.	37.0
5	Hardom	ssp. *manshurica* Enc.	38.5
6	Mandarin	ssp. *manshurica* Enc.	39.0
7	K-5683	ssp. *gracilis*	36.5
8	K-4937	ssp. *slavonica* Enk.	37.0
9	K-6910	*G. ussuriensis*	33.5
10		*G. canescens*	20-25^{+}
11		*G. clandestina*	13-16^{+}
12		*G. tabacina*	20-26^{+}

$^{+}$11S component content varies in individual samples.

Amino acid composition of glycinin samples. The results of the amino acid analysis of glycinin are presented in Table 2. The data allow us to conclude that the content of the individual amino acids is the same in all samples, and the deviations from the mean value do not exceed the experimental error. In general, the results obtained correspond to those of Moreira et al. (1979).

The subunit composition of glycinin of soybean varieties. Fig. 1a demonstrates patterns of glycinin SDS - polyacrylamide gel electrophoresis. One can see that all glycinin patterns, including wild soybean species, are formed by the same acidic and basic subunits with similar electrophoretic characteristics. Fig. 1b shows the results of the isoelectric focusing of glycinin. The pattern reveals noticeable heterogeneity of glycinin polypeptides. Such heterogeneity for isolated glycinin subunits was first reported by Moreira et al. (1981). Similarity in the location of bands representing polypeptides of glycinin from different seed samples is obvious. To reveal the possible differences in subunit composition of glycinin, the acidic subunits were

Table 2. The amino acid composition of glycinin from various seed samples

Sample No.+	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Val	Ile	Leu	Tyr	Phe	His	Lys	Arg	Met
1	10.8	3.7	6.7	18.9	6.8	7.8	4.9	4.7	4.3	7.4	1.9	3.5	2.0	4.8	9.6	1.1
2	11.0	3.8	6.8	19.0	6.7	8.1	4.9	4.6	4.3	7.7	2.3	3.7	2.1	4.8	9.2	1.1
3	11.6	3.8	6.8	19.5	6.9	7.9	5.3	4.6	4.2	6.8	2.0	3.7	2.0	4.5	8.5	1.2
4	11.5	3.8	6.7	19.0	6.5	7.6	5.1	4.7	4.3	7.7	2.2	3.5	1.9	4.4	8.7	1.2
5	11.5	3.8	6.8	19.5	6.8	7.9	5.1	4.7	4.1	6.8	2.1	3.4	2.2	4.7	8.7	1.0
6	11.4	3.6	6.8	20.1	7.1	7.9	4.7	4.7	4.2	7.2	2.2	3.5	2.0	4.2	8.2	1.2
7	11.8	3.8	6.6	20.0	6.1	7.8	5.5	4.9	4.7	8.3	2.3	4.0	1.5	4.0	7.1	1.0
8	11.3	3.8	6.4	18.1	5.2	7.8	5.4	4.9	4.1	7.6	1.9	4.1	1.9	4.9	10.3	1.1
9	11.8	3.8	6.5	20.0	5.9	7.8	5.5	4.9	4.8	8.2	2.0	4.1	1.6	4.2	7.1	1.1

+Sample numbers are the same as in Table 1.

separated by FPLC. The typical chromatographic profile has six major peaks (Fig. 2). Fraction 1 corresponds to basic subunits with the admixture of 4-vinylpyridine. Fractions 1a, 2 and 3 correspond to polypeptides of the electrophoretic mobilities corresponding to those of A_1 subunit (Moreira et al., 1979). Fraction 4 corresponds to A_2 subunit, and fractions 5 and 6 contain subunits A_3 and A_4, respectively. The chromatographic patterns were roughly identical for all samples analyzed. It was of interest to study the subunit structure of glycinin of the wild species of soybean. It appeared that in both *Glycine max* and *Glycine ussuriensis*, the subunit structure of glycinin is very similar (Fig. 2b). Generally, it was found that glycinin from all the seed samples consists of the identical sets of polypeptides.

Conservatism of glycinin subunit composition. The data obtained suggest that the subunit composition of glycinin of cultivated soybean and its probable ancestor is characterized by pronounced conservatism. Staswic et al. (1983) point out that entries from the northern USDA collection also do not exhibit polymorphism. The available data concerning glycinin polymorphism are not numerous and matter rather for scientific than practical aspect of the problem. Thus, according to our data on glycinin structure conservatism, we can scarcely increase the glycinin methionin content by breeding due to the absence of the subunit polymorphism. As glycinin makes up more than 40% of the total protein, the improvement of amino acid composition becomes a quite complicated problem.

The conservatism of glycinin subunit composition seems to depend on its structure, because the storage proteins of other legumes reveal polymorphism. It is also known that other protein components of soybean seeds may be lacking, due to mutations (Orf and Hymowitz, 1979; Orf et al., 1978). The mechanism of glycinin synthesis via high molecular weight precursor (Barton et al., 1982; Epishin and Vinetsky, 1983) limits possible rearrangements, because any subunit substitution will affect both acidic and basic moieties. Besides that, substitution of any pair of subunits may alter the mechanism of assembling of the glycinin molecule. So, the glycinin conservatism probably reflects the strict order of subunit assembling into a glycinin complex.

As it was recently shown by Staswic et al. (1983), wild perennial species of the genus *Glycine* have a slightly different structure of 11S globulin. It is possible that the increase in the 11S protein content and a growth of the seed size are connected with the advantages of glycinin structure, which is

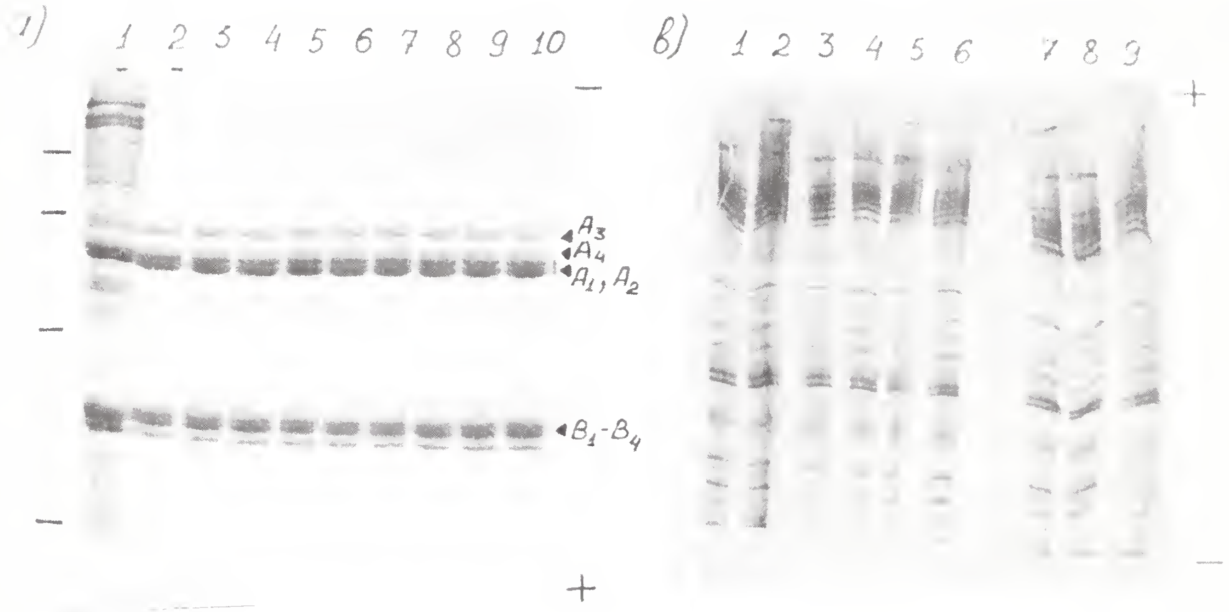

Fig. 1. a) SDS - electrophoresis of purified glycinin in 10% gel. The samples loaded: 1 - Rannaja-10, total protein; 2 - 8-preparations of glycinin; sample numbers correspond to those in Table 1; 9 - glycinin, sample 1; 10 - sample 9. To the left, the position of molecular weight markers are shown (from top to bottom: 67 kD, 45 kD, 25 kD, 12 kD). The bands of glycinin are marked according to Moreira et al. (1979).

b) Isoelectric focusing of purified glycinin in 10% gel. The sample numbers correspond to those in Table 1.

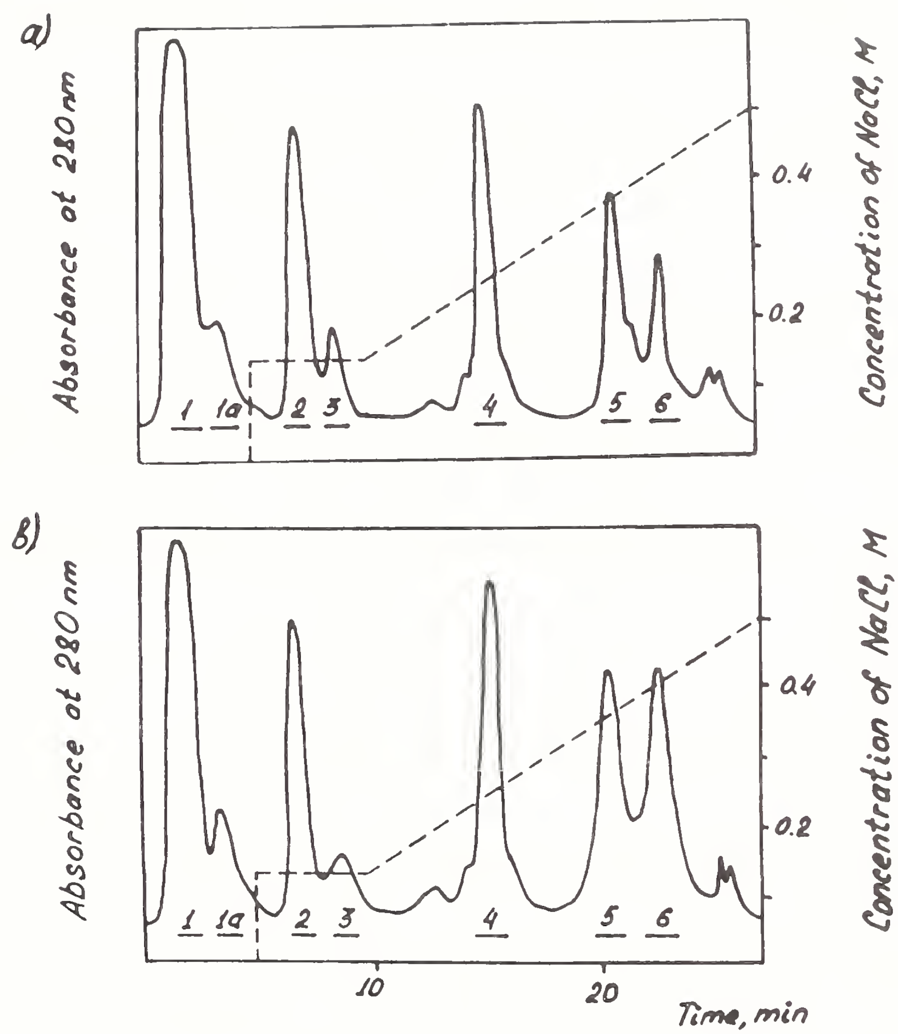

Fig. 2. The chromatographic profiles of reduced glycinin on "Mono Q" column. ______ - the absorbance at 280 nm; ----- - salt concentration. a) Sample 1 (*Glycine max*); b) Sample 9 (*Glycine ussuriensis*).

observed in the cultivated soybean. Evolutionary advantages of this structure seem to have developed in consequence of the genetical isolation during the domestication and as a result of artificial selection.

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Zambia

1) Development of promiscuous soybean varieties.

Zambia was perhaps the first country to initiate research on the development of promiscuous soybean varieties. Promiscuous soybean varieties have the capability of producing effective nodules with the indigenous rhizobia found in the Zambian soils. Two such varieties, 'Magoye' and 'Hernon 147', have already been released in Zambia. These varieties are very popular among small-scale farmers because these can be grown without artificial seed inoculation with *Rhizobium japonicum*.

The development of promiscuous varieties should be viewed as only one possible solution to overcome the problem of inadequate supplies of effective inoculum.

Farmers growing promiscuous varieties can always inoculate their seed before planting if they have sufficient inoculum available. They may even expect a positive yield response. However, if inoculum is not available, use of promiscuous varieties will certainly help in increasing soybean production by small-scale farmers.

During 1977-78 season, one commercially grown soybean variety, Hernon 147, was observed to have nodulated profusely when grown in virgin land without artificial seed inoculation (Javaheri and Nyemba, 1982). These scientists in 1979-80 season observed another breeding line, '71-38', from Queensland, Australia, which nodulated profusely without seed inoculation. This line was later released in April, 1981, under the name Magoye as the second commercial promiscuous soybean variety (Javaheri and Nyemba, 1982). Out of 400 cultivars planted on virgin land without seed inoculation at three different sites in Zambia, 35 to 50 cultivars were observed to be promiscuous and Javaheri (1982) reported that, among these cultivars, some had acceptable agronomic traits. The present investigation was undertaken to evaluate the efficiency of nodulation of selected promiscuous lines with indigenous rhizobia.

Materials and methods. The experiment was planted on virgin land (sandy loam) on December 14, 1983, at the Magoye Regional Research Station, Magoye, Zambia. Magoye is situated at Latitude S, 16°00, Longitude E, 27°36', at an elevation of 1018 meters.

Sixteen promiscuous lines selected from previous studies including two recommended promiscuous soybean varieties, Magoye and Hernon 147, were evaluated with and without inoculation in a split-plot design, varieties being main plots and inoculum sub-plots, with 4 replications. Each main plot consisted of 8 rows, rows being 50 cm apart and 5 m long. Each sub-plot consisted of 4 rows. Yield data were recorded from one of the two central rows (50 cm x 4 m) of each sub-plot. In the two central rows, 50 cm at either end was treated as nonexperimental area. The other central row was used for nodule count. Number of nodules per plant was recorded at 7 weeks after planting and at the end of flowering period for each treatment. Twenty plants were carefully dug for nodule count at each time. Magoye Regional Research Station received 588.6 mm of rainfall during 1983-84 growing season. This rainfall was about 200 mm less than the average rainfall for this site.

Results and discussion. The over-all mean yield without inoculation was 885 kg/ha and with inoculation 913 kg/ha, a slight increase of 3.2% over no inoculation (Table 1).

Table 1. Yield of soybean cultivars with and without inoculum

Cultivar	Without inoculum (kg/ha)	With inoculum (kg/ha)
1. P5	766	932
2. K39	1128	825
3. K49-14	714	761
4. Hernon 147	541	581
5. 49-18	1044	1317
6. K8	864	1013
7. P7	795	174
8. K79	1120	1043
9. K152	1128	1219
10. K53	1112	1205
11. M27	539	524
12. M30	1116	1296
13. K134	944	963
14. TG x 326 - 034D	609	591
15. TG x 297 - 192C	648	840
16. Magoye	1092	746
$\overline{X}$	885	913

Without inoculation, cultivar Magoye produced 1092 kg/ha, and five other lines, namely K39 (1128 kg/ha), K152 (1128 kg/ha), K79 (1120 kg/ha), M30 (1116 kg/ha) and K53 (1112 kg/ha), produced better yields than Magoye. Out of these 5 lines, K39 and K79 responded negatively to inoculation as far as yield is concerned. Negative response to yield in cultivar Magoye was also observed (Table 1). The yield levels of all cultivars in this study are very low. This is because the experiment was conducted on virgin land that has lower fertility status as compared to cultivated land.

The number of nodules per plant, 7 weeks after planting and at the end of flowering period, were slightly more with inoculation than without inoculation (Table 2). At 7 weeks after planting under no inoculation treatment, the number of nodules/plant for K79, M30, K53, K39, K152 and Magoye were 14.8, 10.5, 9.3, 7.0, 5.0 and no nodules, respectively, but at the end of the flowering period, the number of nodules per plant decreased slightly in case of K39, K79, K53 and M30. However, a large increase in case of Magoye (from 0 to 11.3 nodules per plant) was observed during the same period. The number of nodules per plant also increased (from 5.0 to 6.7) for K152 under no inoculation treatment.

Table 2. Number of nodules per plant

Cultivar	7 weeks after planting		At the end of flowering	
	Without inoculum	With inoculum	Without inoculum	With inoculum
1. P5	3.5	1.8	4.4	6.6
2. K39	7.0	13.8	6.3	6.6
3. 49-14	7.0	9.0	5.2	4.4
4. Hernon 147	3.0	3.8	2.5	3.2
5. 49-18	7.3	11.8	11.9	15.4
6. K8	5.0	1.8	9.4	8.4
7. P7	3.8	9.0	5.3	5.8
8. K79	14.8	2.3	11.8	13.9
9. K152	5.0	3.5	6.7	8.7
10. K53	9.3	12.8	7.7	9.6
11. M27	3.3	11.3	4.8	4.2
12. M30	10.5	1.5	9.1	11.4
13. K134	1.5	0.3	5.5	6.8
14. TG x 326 - 034D	0	0	0.6	6.0
15. TG x 297 - 192C	5.0	1.3	1.7	3.1
16. Magoye	0	7.8	11.3	8.7
$\overline{X}$	5.4	5.7	6.5	7.7

All six cultivars discussed are late maturing and take 137 to 142 days to mature. These six cultivars are now being evaluated in the Zambian promiscuous soybean variety trial at three different locations in Zambia.

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